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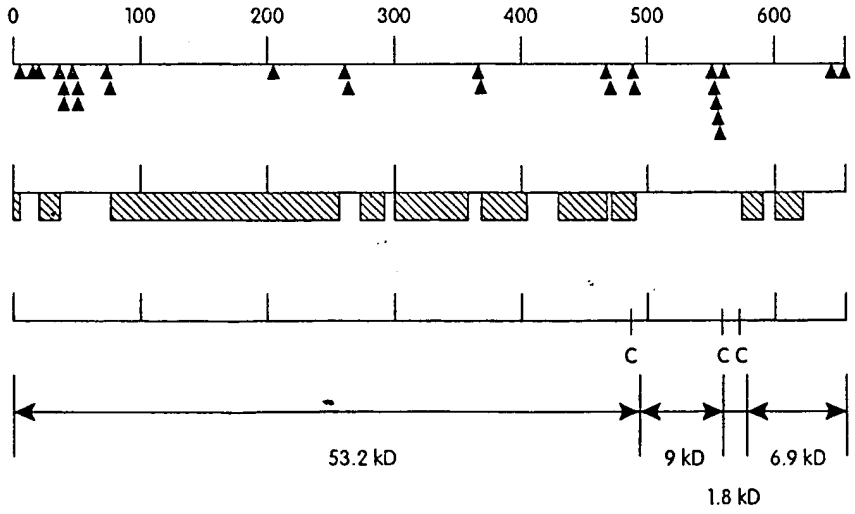

(57) Abstract

Disclosed are genetic sequences and their encoded amino acid sequences for two interior nuclear matrix proteins useful as markers of malignant cell types. Primary and secondary structure analysis of the proteins is presented as well as means for their recombinant production, and compositions and methods for the use of these markers in clinical assays and cancer therapies.

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Novel Malignant Cell Type Markers of the Interior Nuclear Matrix

Background of the Invention

All eucaryotic cells, both plant and animal, have a nucleus surrounded by the cell cytoplasm. The nucleus contains the cellular DNA complexed with protein and termed chromatin. The chromatin, with its associated proteins, constitutes the major portion of the nuclear mass and is organized by the internal protein skeleton of the nucleus, referred to here as the nuclear matrix (NM). The nuclear matrix also is defined as the nuclear structure that remains following removal of the chromatin by digestion with DNase I and extraction with high salt. This skeletal nuclear structure further is characterized by the "interior nuclear matrix" (INM) and the bounding nuclear pore-lamina complex.

Diverse studies have implicated the NM in a wide variety of nuclear functions fundamental to the control of gene expression (For a general review see, for example, Fey et al. (1991) Crit. Rev. Euk. Gene Express 1:127-143). In particular, as described in U.S. Pat. Nos. 4,882,268 and 4,885,236, it is now known that certain nuclear matrix proteins, specifically interior nuclear matrix proteins, are useful as marker proteins for identifying cell types. For example, the presence and abundance of particular INM proteins have been shown to be characteristic of specific cell types and can be used to identify the tissue of origin of a cell or cell fragment present in a sample. One particularly important application of this discovery is the use of marker INM proteins in evaluating metastatic

tissue. It is also known that the expression of certain INM proteins is altered in malignant or otherwise dysfunctional cells. The altered expression pattern of these proteins in maligrant and/or dysfunctioning cells also makes the proteins and nucleic acids encoding the proteins useful as marker proteins, alone or in combination, for diagnostic purposes and for evaluating tissue viability. US Pat. Nos. 4,882,268 and 4,885,236, issued 11/21/89 and 12/5/89, respectively, to Penman and Fey, disclose a method for selectively extracting insoluble INM proteins and their associated nucleic acids from cells or cellular debris and distinguishing the expression pattern of these proteins in a particular cell type by displaying the proteins on a two-dimensional electrophoresis gel. In addition, it recently has been discovered that INM proteins or protein fragments also may be released in soluble form from dying cells. (US Application Serial No. 785,804, filed October 31, 1991.)

To date, molecular characterization of the specific proteins of the NM, particularly the INM, remain poorly defined due to the low abundance of these proteins in the cell and their generally insoluble character. The ability to isolate and characterize specific nuclear matrix proteins and the genetic sequences encoding them at the molecular level is anticipated to enhance the use of these proteins and their nucleic acids as marker molecules, and to enhance elucidation of the biological role of these proteins in vivo.

It is an object of this invention to provide genetic sequences encoding INM proteins useful as markers of malignant cell types. Another object is to provide enhanced means for identifying these proteins and their nucleic acids, including RNA transcripts, in samples. Yet another object of this invention is to provide compositions for use in diagnostic and other tissue evaluative procedures. Still another object is to provide genetic and amino acid sequences useful as target molecules in a cancer therapy. These and other objects and features of the invention will be apparent from the description, figures and claims which follow.

Summary of the Invention

Molecular characterization data, including DNA sequence data, for two INM proteins now have been derived from an expression library, using monoclonal antibodies for these proteins. The proteins, designated herein as MT1 and MT2, are present at elevated levels in malignant tissue and extracellular fluids. Accordingly, the proteins and the genetic sequences encoding them are thought to be useful as marker molecules for identifying tissue tumorgenesis in cell or body fluid samples.

Full or partial clones of the genes encoding these proteins now have been isolated, and the DNA sequence, reading frames and encoded amino acid sequences of these DNAs determined. The DNA sequence for MT2 corresponds to the sequence disclosed by Yang, et al. (1992) J. Cell Biol. 116:1303-1317, and Compton et al. (1992) J. Cell Biol. 116:1395-1408, referred to therein as NuMA. The nucleic acid (and the encoded amino acid sequence) described herein for MT1 has not been described previously and also constitutes a novel sequence sharing little sequence homology with those sequences known in the art. In addition, MT1 has been subcloned into an expression vector, and the protein expressed as a cleavable fusion protein in E. coli. Both the MT1 and MT2 (NuMA) proteins are distributed throughout the nucleus (with the exception of the nucleolus) in non-mitotic cells, and localize to the spindle during mitosis, as determined immuno-fluoresence.

The genetic sequences described herein provide a family of proteins for each of the proteins MT1 and MT2, including allelic and species variants of MT1 and MT2. The family of proteins include these proteins produced by expression in a host cell from recombinant DNA, the DNA itself, and the host cells harboring and capable of expressing these nucleic acids. The recombinantly produced proteins may be isolated using standard methodologies such as affinity chromatography to yield substantially pure proteins. As used herein, "substantially pure" is understood to mean substantially free of undesired, contaminating proteinaceous material.

The family of proteins defined by MT1 includes proteins encoded by the nucleic acid sequence of Seq. ID No. 1, including analogs thereof. As used herein, "analog" is understood to include allelic and species variants, and other naturally-occurring and engineered mutants. These variants include both biologically active and inactive forms of the protein. Particularly envisioned are DNAs having a different preferred codon usage, those having "silent mutations" of the DNA of Seq. ID No.1, wherein the changes in the genetic sequence do not affect the encoded amino acid sequence, and DNAs encoding "conservative" amino acid changes, as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol. 5, Supp. 3, pp 345-362 (M.O. Dayoff, ed., Nat'l Biomed. Research Foundation, Washington, D.C. 1979.)

Accordingly, the nucleic acids encoding the protein family of MT1 may be defined as those sequences which hybridize to the DNA sequence of Seq. ID No.1 under stringent hybridization conditions. As used herein,

stringent hybridization conditions are as defined in Molecular Cloning: A Laboratory Manual, Maniatis, et al. eds., Cold Spring Harbor Press, 1985, e.g.: hybridization in 50% formamide, 5x Denhardt's Solution, 5 x SSPE, 0.1% SDS and 100 μg/ml denatured salmon sperm, and washing in 2 x SSC, 0.1% SDS, at 37°C, and 1 x SSC, 0.1% SDS at 68°C.

The family of proteins defined by MT2 includes proteins encoded by the nucleic acid sequence of Seq. ID No. 3, including analogs thereof, including allelic and species variants, and other naturally-occurring and engineered mutants. These variants include both biologically active and inactive forms of the protein. Particularly envisioned are DNAs having silent mutations, other preferred codon usages, and DNAs encoding conservative amino acid changes. The nucleic acids encoding the protein family of MT2 of this invention may be defined as those sequences which hybridize with the DNA sequence of Seq. ID No. 3 under stringent hybridization conditions.

In another aspect, the invention provides nucleic acid fragments ("oligonucleotides" or "oligomers") which hybridize to genetic sequences encoding MT1, but which do not necessarily encode functional proteins themselves. The oliognucleotides include probes for isolating genetic sequences encoding members of the MT1 family of proteins from a cDNA or genomic DNA library, and/or for identifying genetic sequences naturally associated with the MT1 protein coding sequence e.g., sequences lying upstream or downstream from the coding sequences. For example, where the nucleic acid fragment is to be used as a probe to identify other members of the MT1 family, the nucleic acid fragment

may be a degenerate sequence as described in Molecular Cloning: A Laboratory Manual, Maniatis, et al. eds., Cold Spring Harbor Press, 1985, designed using the sequence of Seq. ID No.1 as a template. Accordingly, the oligonucleotide or nucleic acid fragment may comprise part or all of the DNA sequence of Seq. ID No. 1, or may be a biosynthetic sequence based on the DNA sequence of Seq. ID No. 1. The oligonucleotide preferably is suitably labelled using conventional labelling techniques.

The oligonucleotides also include sequences which hybridize with the mRNA transcript encoding the MT1 protein. These complementary sequences are referred to in the art and herein as antisense sequences. Antisense sequences may comprise part or all of the sequence of Seq. ID No. 1, or they may be biosynthetic sequences designed using the sequence of Seq. ID No. 1 as a template.

In still another aspect, the invention provides oligonucleotides which hybridize to the genetic sequences encoding members of the MT2 protein family. The fragments include antisense sequences and sequences useful as probes for identifying members of the MT2 family and/or for identifying associated noncoding sequences. The hybridizing nucleic acids may comprise part or all of the sequence of Seq. ID No. 3 or may be biosynthetic sequences designed using the DNA sequence of Seq. ID No. 3 as a template, preferably suitably labelled using conventional techniques.

The genetic sequences identified herein encode proteins identified as marker proteins indicative of a malignancy or other cellular dysfunction in a tissue. Thus, in another aspect, the invention provides compositions for obtaining antibodies useful for detecting cancer marker proteins in a sample using the proteins described herein in combination with a suitable adjuvant. In another aspect, the invention provides genetic templates for designing sequences which hybridize specifically with the mRNA transcripts encoding these proteins. In still another aspect, the invention provides isolated DNA sequences for use in expressing proteins and protein fragments for the design of binding proteins, including antibodies, which interact specifically with an epitope on MT1 or MT2. The invention also provides methods for evaluating the status of a tissue using the genetic sequences described herein, and the marker proteins encoded by them. Finally, the invention provides methods for treating a malignancy in an individual using these marker proteins, or the genetic sequences encoding them, as target molecules to inhibit or disable the cell's ability to undergo cell division.

Brief Descriptions of the Drawings

Fig. 1A-1D is a schematic representation of the amino acid sequence of MT1 of Seq. ID No.1, showing:

Fig. 1A: the location of the proline residues;
Fig. 1B: the areas defined as ∝-helices within the sequence;
Fig. 1C: the location of the cysteine residues; and
Fig. 1D: the sites of cleavage by NTCB;

Fig. 2A-2B is a schematic representation of the amino acid sequence of MT2 of Seq. ID No.3, showing:

Fig. 2A: the location of proline residues; and
Fig. 2B: the areas defined as ∝-helices within the sequence;

Fig. 3: lists the levels of body fluid-soluble MT2 and MT2-associated protein quantitated in various normal and malignant tissue sample supernatants; and

Fig. 4: lists the levels of body fluid-soluble MT2 and MT2-associated protein quantitated in sera isolated from cancer patients and normal blood donors.

Detailed Description

In an attempt to characterize INM proteins useful as malignant cell markers in biological assays, the genetic sequences encoding two INM proteins, herein referred to as MT1 and MT2, now have been identified and characterized. DNA sequences encoding these proteins now have been cloned by probing expression libraries using monoclonal antibodies raised against the isolated INM proteins MT1 and MT2. The proteins were isolated from malignant cells essentially following the method of Penman and Fey, described in U.S. Pat. Nos. 4,882,268 and 4,885,236, the disclosures of which are herein incorporated by reference. The cloned DNAs, then were sequenced and their reading frames identified and analyzed. The genetic sequence encoding MT2 also has been disclosed by others (Yang, et al. (1992) J. Cell Biol. 116:1303-1317 and Compton et al. (1992) J. Cell. Biol. 116:1395-1408), and is referred to by them as "NuMA". Comparison of MT1 and MT2 (NuMA) with other sequences in the art indicate that the sequences encoding these proteins constitute sequences sharing little homology with previously described sequences.

MT1 also has been expressed as a cleavable fusion protein in E. coli and compared with the protein isolated from mammalian cells. Anti-MT1 antibodies raised against the natural-sourced MT1 protein also crossreact with the recombinantly produced protein.

Both the natural-sourced and recombinantly produced proteins have the same apparent molecular weight when analyzed by SDS-PAGE (90kD), equivalent pI values (5.4), and both proteins show the same cleavage pattern when cleaved with 2-nitro-3-thiocyanobenzoic acid (NTCB, see infra.)

Immunolocalization data on MT1 indicates that MT1 protein is distributed within the INM in non-mitotic cells as discrete punctate foci, nonuniformly distributed throughout the nucleoplasm of the INM. Specifically, the foci are present in the interchromatinic regions of the nucleus and are distributed in a stable association that remains after chromatin extraction, as is anticipated for an interior nuclear matrix protein. In addition, MT1 foci are excluded from the nucleolus and the nuclear lamina. Moreover, during mitosis, the distribution of MT1 changes and MT1 becomes aligned in a stellate or star-shaped pattern at the spindle of the dividing cell. The protein does not co-localize with the chromosomes, suggesting that MT1 may play a structural role during mitosis. The immunolocalization data is consistent with the MT1 amino acid sequence analysis data which fails to find structural homology with any known DNA binding motifs, such as the "leucine zipper."

While the MT2 (NuMA) protein has not yet been recombinantly expressed, the predicted molecular weight of 238 kDa for this protein, calculated from the predicted amino acid sequence (see Seq. ID No. 3), agrees with that of the natural-sourced material.

Immunolocalization studies on MT2 (NuMA) indicate that it also forms punctate foci located throughout the nucleoplasm of the non-mitotic cell, and also is excluded from the nucleolus. During mitosis the protein appears to migrate to the spindle poles of the dividing cell. The primary sequence appears to suggest a coiled-coil motif for the folded protein (Compton, et al. (1992) J. Cell Biol. 116:1395-1408; Yang, et al. (1992) J. Cell Biol. 116:1303-1317.)

I. How to Use

The nucleic acids disclosed herein encode proteins originally identified as marker proteins useful for identifying cell malignancies or other cell abnormalties. Specifically, significantly elevated levels of these proteins are detected in malignant cells and in extracellular fluids, e.g., sera, of cancer patients. (See PCT publication WO93/09437 and infra.) For example, the presence and/or abundance of these proteins or their transcripts in a sample containing cells or cell nuclear debris may be used to determine whether a given tissue comprises malignant cells or cells having other abnormalities, such as chromosomal abnormalities. The sample may be an exfoliated cell sample or a body fluid sample, e.g., a sample comprising blood, serum, plasma, urine, semen, vaginal secretions, spinal fluid, saliva, ascitic fluid, peritoneal fluid, sputum, tissue swabs, and body exudates such as breast exudate.

In addition, because INM proteins are released in soluble form from dying cells, the marker molecules may be used to evaluate the viability of a given tissue. For example, the marker proteins may be used to

evaluate the status of a disease or the efficacy of a therapeutic treatment or procedure, by monitoring the release of these marker molecules into a body fluid over a period of time. Particularly useful body fluids include blood, serum, plasma, urine, semen, vaginal secretions, spinal fluid, saliva, ascitic fluid, peritoneal fluid, sputum, tissue swabs, and body exudates such as breast exudate. Methods for performing these assays are disclosed in U.S. Pat. Nos. 4,882,268 and 4,885,236 and in co-pending U.S. application Serial Nos. 214,022, filed June 30, 1988 and U.S. application Serial No. 785,804, filed October 31, 1991, the disclosures of which all are herein incorporated by reference.

All of these assays are characterized by the following general procedural steps:

1) detecting the presence and/or abundance of the marker protein or its transcript in "authentic" or reference samples;

2) detecting the presence and/or abundance of the marker protein or its transcript in the sample of interest; and

3) comparing the quantity of marker protein or its transcript in the sample of interest with the quantity present in the reference sample.

Where the assay is used to monitor tissue viability, the step of detecting the presence and abundance of the marker protein or its transcript in samples of interest is repeated at intervals and these values then are compared, the changes in the detected

concentrations reflecting changes in the status of the tissue. Where the assay is used to evaluate the efficacy of a therapy, the monitoring steps occur following administration of the therapeutic agent or procedure (e.g., following administration of a chemotherapeutic agent or following radiation treatment.)

It is not required that the selected marker protein or transcript be totally unique, in the sense that the particular INM marker molecule is present in the target cell type and in no other. Rather, it is required that the marker molecule have a signal to noise ratio high enough to discriminate the preselected cell type in samples for which the assay is designed. For example, MT1 and MT2 proteins are useful as proteins indicating the presence of malignancy in cell samples because of their elevated expression levels in malignant cells, even though the proteins, or close analogs thereof, may be present commonly in nonmalignant cell types.

A brief description of general protein and nucleic acid assay considerations follows below. Details of particular assay conditions may be found in the assay references described above and incorporated herein by reference, and in published protocols well known in the art and readily available.

A. Protein Assays

Characterization of the MT1 and MT2 proteins at the molecular level as described herein allows one to characterize the proteins structurally and biochemically. Accordingly, following the disclosure of these genetic sequences and their encoded amino acid

sequences, preferred binding epitopes may be identified which may be used to enhance assay conditions. For example, binding proteins may be designed which have enhanced affinity for the marker protein produced by particular cell types or as a function of particular malignancies. Similarly, binding proteins may be designed which bind preferentially to protein fragments released from dying cells. In addition, structural and/or sequence variations between proteins produced in normal and abnormal tissue now may be investigated and used to advantage. The genetic sequences may be manipulated as desired, e.g., truncated, mutagenized or the like, using standard recombinant DNA procedures known in the art, to obtained proteins having desired features useful for antibody production.

As will be appreciated by those skilled in the art, any means for specifically identifying and quantifying a marker protein of interest is contemplated. The currently preferred means for detecting a protein of interest in a sample is by means of a binding protein capable of interacting specifically with the marker protein. Labelled antibodies or the binding portions thereof in particular may be used to advantage. The antibodies may be monoclonal or polyclonal in origin, or may be biosynthetically produced. The amount of complexed marker protein, e.g., the amount of marker protein associated with the binding protein, then is determined using standard protein detection methodologies well described in the art.

A.1. Immunoassays

A variety of different forms of immunoassays currently exist, all of which may be adapted to detect and quantitate INM proteins and protein fragments. For exfoliated cell samples, as an example, the cells and surrounding fluid are collected and the INM proteins selectively isolated by the method of Penman and Fey, described in U.S. Pat. Nos. 4,882,268 and 4,885,236. These proteins then preferably are separated by two-dimensional gel electrophoresis and the presence of the marker protein detected by standard Western blot procedures.

For serum and other fluid assays where the marker proteins and/or protein fragments to be detected exist primarily in solution, one of the currently most sensitive immunoassay formats is the sandwich technique. In this method, as described in PCT publication WO93/09437 and which has a precision typically of ± 5%, two antibodies capable of binding the analyte of interest generally are used: e.g., one immobilized onto a solid support, and one free in solution, but labeled with some easily detectable chemical compound. Examples of chemical labels that may be used for the second antibody include radioisotopes, fluorescent compounds, and enzymes or other molecules which generate colored or electrochemically active products when exposed to a reactant or enzyme substrate. When samples containing the marker protein or protein fragment are placed in this system, the marker protein binds to both the immobilized antibody and the labelled antibody. The result is a "sandwich" immune complex on the support's surface. The complexed protein is detected by washing

away nonbound sample components and excess labeled antibody, and measuring the amount of labeled antibody complexed to protein on the support's surface. The sandwich immunoassay is highly specific and very sensitive, provided that labels with good limits of detection are used. A detailed review of immunological assay design, theory and protocols can be found in numerous texts in the art, including Practical Immunology, Butt, W.R., ed., Marcel Dekker, New York, 1984.

In general, immunoassay design considerations include preparation of antibodies (e.g., monoclonal or polyclonal) having sufficiently high binding specificity for their antigen that the specifically-bound antibody-antigen complex can be distinguished reliably from nonspecific interactions. As used herein, "antibody" is understood to include other binding proteins having appropriate binding affinity and specificity for the marker protein. The higher the antibody binding specificity, the lower the antigen concentration that can be detected. Currently preferred binding specificity is such that the binding protein has a binding affinity for the marker protein of greater than about 10^5 M^{-1}, preferably greater than about $10^7 M^{-1}$.

Antibody binding domains also may be produced biosynthetically and the amino acid sequence of the binding domain manipulated to enhance binding affinity with a preferred epitope. Identification of the genetic sequences for MT1 and MT2 can be used to advantage in the design and construction of preferred binding proteins. For example, a DNA encoding a preferred epitope may be recombinantly expressed and

used to select an antibody which binds selectively to the eptiope. The selected antibodies then are exposed to the sample under conditions sufficient to allow specific binding of the antibody to its specific nuclear matrix protein or protein fragment, and the amount of complex formed then detected. Specific antibody methodologies are well understood and described in the literature. A more detailed description of their preparation can be found, for example, in Practical Immunology, Butt, W.R., ed., Marcel Dekker, New York, 1984.

The choice of tagging label also will depend on the detection limitations desired. Enzyme assays (ELISAs) typically allow detection of a colored product formed by interaction of the enzyme-tagged complex with an enzyme substrate. Alternative labels include radioactive or fluorescent labels. The most sensitive label known to date is a chemiluminescent tag where interaction with a reactant results in the production of light. Useful labels include chemiluminescent molecules such as acridium esters or chemiluminescent enzymes where the reactant is an enzyme substrate. When, for example, acridium esters are reacted with an alkaline peroxide solution, an intense flash of light is emitted, allowing the limit of detection to be increased 100 to 10,000 times over those provided by other labels. In addition, the reaction is rapid. A detailed review of chemiluminescence and immunoassays can be found in Weeks, et al., (1983) Methods in Enzymology 133:366-387. Other considerations for fluid assays include the use of microtiter wells or column

immunoassays. Column assays may be particularly advantageous where rapidly reacting labels, such as chemiluminescent labels, are used. The tagged complex can be eluted to a post-column detector which also contains the reactant or enzyme substrate, allowing the subsequent product formed to be detected immediately.

A.2. Antibody Production

The proteins described herein may be used to raise antibodies using standard immunological procedures well known and described in the art. See, for example, Practical Immunology, Butt, N.R., ed., Marchel Dekker, NY, 1984. Briefly, an isolated INM protein produced, for example, by recombinant DNA expression in a host cell, is used to raise antibodies in a xenogenic host. Preferred antibodies are antibodies that bind specifically to an epitope on the protein, preferably having a binding affinity greater than $10^5 M^{-1}$, most preferably having an affinity greater than $10^7 M^{-1}$ for that epitope. For example, where antibodies to a human INM protein, e.g. MT1 or MT2 is desired, a suitable antibody generating host is a mouse, goat, rabbit, guinea pig, or other mammal useful for generating antibodies. The protein is combined with a suitable adjuvant capable of enhancing antibody production in the host, and injected into the host, for example, by intraperitoneal administration. Any adjuvant suitable for stimulating the host's immune response may be used to advantage. A currently preferred adjuvant is

Freund's complete adjuvant (an emulsion comprising killed and dried microbial cells, e.g., from Calbiochem Corp., San Diego, or Gibco, Grand Island, NY). Where multiple antigen injections are desired, the subsequent injections comprise the antigen in combination with an incomplete adjuvant (e.g. cell-free emulsion).

Polyclonal antibodies may be isolated from the antibody-producing host by extracting serum containing antibodies to the protein of interest. Monoclonal antibodies may be produced by isolating host cells that produce the desired antibody, fusing these cells with myeloma cells using standard procedures known in the immunology art, and screening for hybrid cells (hybridomas) that react specifically with the INM protein and have the desired binding affinity.

Provided below is an exemplary protocol for monoclonal antibody production, which is currently preferred. Other protocols also are envisioned. Accordingly, the particular method of producing antibodies with the cancer marker protein compositions of this invention, is not envisioned to be an aspect of the invention. Also described below are exemplary sandwich immunoassays and dot blot assays useful for detecting and/or quantitating marker proteins in a sample. Other means for detecting marker proteins, particularly MT1, MT2 and their analogs, including protein fragments and naturally-occurring variants, also are envisioned. These other methods are well-known and described in the art.

Exemplary antibody production protocol: Balb/c by J mice (Jackson Laboratory, Bar Harbor, ME) are injected intraperitoneally with purified INM protein (e.g., MT1) purified from the human cervical cell line CaSki, every 2 weeks for a total of 16 weeks. The mice are injected with a single boost 4 days prior to sacrifice and removal of the spleen. Freund's complete adjuvant (Gibco, Grand Island) is used in the first injection, incomplete Freund's in the second injection; subsequent injections are made with saline. Spleen cells (or lymph node cells) then are fused with a mouse myeloma line, e.g., using the method of Kohler and Milstein (1975) Nature 256:495, the disclosure of which is incorporated herein by reference, and using polyethylene glycol (PEG, Boehringer Mannheim, Germany). Hybridomas producing antibodies that react with nuclear matrix proteins then are cloned and grown as ascites. Hybridomas are screened by nuclear reactivity against the cell line that is the source of the immunogen, and by tissue immunochemistry using standard procedures known in the immunology art. Detailed descriptions of screening protocols, ascites production and immunoassays also are disclosed in PCT publication WO93/09437.

Exemplary Assays:

A. Sandwich Immunoassay (ELISA)

A standard immunoassay can be performed to generate dose response curves for antigen binding, for cross reactivity assays, and for monitoring assays. The data

is generated with a standard preparation of NM antigen, and is used as the reference standard when body fluids are assayed. In these examples both ELISAs and radioummunoassays were performed.

1. Immunoassay (Well Assay)

Microtitre plates (Immulon II, Dynatech, Chantilly, VA) are coated with purified antibody at 5 to 15ug/ml in PBS at pH 7.4 for 1hr or overnight and then washed 3 x with 300μl PBS. The plates then are blocked with 10% normal goat serum in PBS for 1hr at room temp and washed 3 x with 300μl of PBS. An exemplary protocol follows.

Here, samples are assayed by pipetting 100μl of sample per well, and incubating for 1hr at RT. The wells were washed with 3 x 300μl PBS. 100μl of 1.25 to 10μg/ml of a biotinylated antibody added to each well, incubated for 1hr at RT and washed with 3 x 300μl of PBS. 100μl of a 1:1000 dilution of streptavidin-horseradish peroxidase conjugate (The Binding Site Ltd., Birmingham, UK) added to each well and incubated for 1hr and then washed with PBS. 100μl of peroxidase substrate (citrate, phosphate, OPD-H_2O_2) then is added to each well and incubated for 20min. The reaction is stopped by adding 50μl of 1M H_2SO_4 to the wells. The optical density is read on a plate reader at 490nm.

Concentrations of antigen are determined by preparing a reference concentration of antigen and preparing a standard dilution curve to compare with the unknown samples.

2. IRMA (Immunoradiometric Assay)

(a) Iodination of Streptavidin.

10μg of streptavidin (Sigma, Inc., Cincinnati) in 2μl of 0.05M phosphate pH 7.4 was added to 10μl of 0.25M phosphate pH 7.4 in a microcentrifuge tube and 1mCi of ^{125}I (NEN-DUPONT, Wilmington, DE) in 10μl is added. Immediately 10μl of 100mg chloramine-T trihydrate (Sigma, Inc.) in 50ml of distilled water is added, mixed, and reacted for 25sec. The reaction then is stopped by mixing for 20sec with 50μl of 40mg Cysteamine (2-mercaptoethlyamine)(Sigma, Inc.) and 5mg KI in 50ml of 0.05M phosphate pH 7.4. 0.5ml of 1% BSA in PBS pH 7.4 added and the material fractionated on a 10ml sephadex G-100 column (Pharmacia, Sweden) pre-equilibrated with the BSA PBS buffer. 30 by 0.5ml fractions are collected and 10μl diluted to 1ml of the BSA/PBS buffer for each fraction. 100μl of the diluted fraction is counted on a LKB gamma counter set for ^{125}I. The specific activity is calculated and routinely falls between 85 to 100uCi/ug. The mid fractions of the protein peak then are used in the sandwich immunoassay.

(b) Sandwich Radioimmunoassay.

The microtitre breakaway wells (Immulon II Removawell strips, Dynatech, Chantilly, Va) are coated and blocked as in the ELISA assay. The samples, standard or sera, are routinely measured by incubating 100μl in the wells for 1hr at RT washing on a plate washer with 3 x 300μl of PBS and then incubated with the biotinylated antibody (2-10μg/ml in 10% goat serum) for 1hr at RT and washed again. The bound biotinylated antibody is detected with the ^{125}I-streptavidin.

200,000 to 300,000 cpm (77% counter efficiency) in 100μl is added to each well and incubated for 1hr at RT and washed again. The bound fraction is detected by counting the radioactivity in an LKB gamma counter. The concentration can be determined by comparing the counts obtained against a reference preparation.

B. Dot Blot Detection of NM.

Antibody reactivity with NM proteins can be assessed by dot blot detection assays, using standard methodologies and apparatus (e.g.,Schleicher & Schuell). Nitrocellulose membranes are soaked in Tris buffered saline, (TBS, 50mM TRIS, 150mM NaCl, pH 7.6) and NM preparation applied at varying concentrations of protein to a series of wells and incubated for 1hr at room temperature (e.g., T-47D NM supernatant at 10μg/ml, 1μg/ml and 100ng/ml). The blocked wells then are washed with 2 x 200μl of TBS and then blocked with 100μl 10% normal goat serum in TBS for 1hr at room temperature. The blocked wells then are washed again with 2 x 200μl of TBS and 100μl of culture supernatant containing nuclear reactive antibody to be tested is added to their respective wells and incubated for 1hr at room temperature. The wells then are washed with 2 x 200μl of TBS and 100μl of a dilution series of alkaline phosphatase conjugated goat anti-mouse IgG (Bio-Rad, Richmond, CA) (e.g., 1:1000, 1: 5000, or 1:10000) added to the relevant wells and incubated for 1hr. The wells then are washed with 2 x 200μl of TBS followed by addition of enzyme substrate (BCIP/NBT, Kirkgaard and Perry, Gaithersburg, MD, e.g., 100 μl) in Tris buffer containing Levamisole (Vector, Inc., Corpus Christi, TX.) A fifteen minute incubation generally is sufficient. The reaction can be stopped by washing with distilled water and the product detected.

B. Nucleic Acid Assays

The status of a tissue also may be determined by detecting the quantity of transcripts encoding these cancer marker proteins. The currently preferred means for detecting mRNA is by means of northern blot analysis using labeled oligonucleotides e.g., nucleic acid fragments capable of hybridizing specifically with the transcript of interest. The currently preferred oligonucleotide sequence is a sequence encoding a complementary sequence to that of at least part of the transcript marker sequence. These complementary sequences are known in the art as "antisense" sequences. The oligonucleotides may be oligoribonucleotides or oligodeoxyribonucleotides. In addition, oligonucleotides may be natural oligomers composed of the biologically significant nucleotides, i.e., A (adenine), dA (deoxyadenine), G (guanine), dG (deoxyguanine), C (cytosine), dC (deoxycytosine), T (thymine) and U (uracil), or modified oligonucleotide species, substituting, for example, a methyl group or a sulfur atom for a phosphate oxygen in the inter-nucleotide phosohodiester linkage. (see, for example, Section I.C, below.) Additionally, the nucleotides themselves, and/or the ribose moieties may be modified.

The sequences may be synthesized chemically, using any of the known chemical oligonucleotide synthesis methods well described in the art. For example, the oligonucleotides are advantageously prepared by using any of the commercially available, automated nucleic acid synthesizers. Alternatively, the oligonucleotides may be created by standard recombinant DNA techniques, by, for example, inducing transcription of the noncoding strand. For example, the DNA sequence

encoding a marker protein may be inverted in a recombinant DNA system, e.g., inserted in reverse orientation downstream of a suitable promoter, such that the noncoding strand now is transcribed.

Useful hybridizing oligonucleotide sequences include any sequences capable of hybridizing specifically to the MT1 or MT2 primary transcripts. Accordingly, as will be appreciated by those skilled in the art, useful sequences contemplated include both sequences complementary to the DNA sequences provided in Seq. ID No. 1 (MT1) or Seq. ID No. 3 (MT2) which correspond to the protein coding regions, as well as sequences complementary to transcript sequences occurring further upstream or downstream from the coding sequence (e.g., sequences contained in, or extending into, the 5'- and 3' untranslated regions). Representative antisense sequences are described in Seq. ID Nos. 5 and 6. Seq. ID No. 5 describes a sequence complementary to the first 100 nucleotides of the MT1 protein coding sequence (compare Seq. ID Nos. 1 and 5) as well as the 53 nucleotide sequence occurring upstream of the initiation codon. The complementary nucleotides to the initiation codon occur at positions 298-300 in Seq. ID No. 5. Similarly, Seq. ID No. 6 describes a sequence complementary to the first 100 nucleotides of the MT2 protein coding sequence (compare Seq. ID Nos. 3 and 6), as well as the 48 nucleotide sequence occurring upstream of the initiation codon. The complementary nucleotides to the initiation codon occur at positions 298-300 in Seq. ID No. 6. Useful oligomers may be created based on part or all of the sequences in Seq. ID No. 5 and 6. However, as will be appreciated by those skilled in the art, other useful sequences which hybridize to other

regions of the transcript readily are created based on the sequences presented in Seq. ID Nos. 1 and 3 and/or additional, untranslated sequences, such as are disclosed for MT2 (NuMA) in Compton et al. and Yang et al.

While any length oligonucleotide may be utilized to hybridize an mRNA transcript, sequences less than 8-15 nucleotides may be less specific in hybridizing to target mRNA. Accordingly, oligonucleotides typically within the range of 8-100 nucleotides, preferably within the range of 15-50, nucleotides are envisioned to be most useful in standard RNA hybridization assays.

The oligonucleotide selected for hybridizing to the INM transcript, whether synthesized chemically or by recombinant DNA, then is isolated and purified using standard techniques and then preferably labelled (e.g., with ^{35}S or ^{32}P) using standard labelling protocols.

A sample containing the marker transcript of interest then is run on an electrophoresis gel, the dispersed nucleic acids transferred to a nitrocellulose filter and the labelled oligonucleotide exposed to the filter under suitable hybridizing conditions, e.g. 50% formamide, 5 X SSPE, 2 X Denhardt's solution, 0.1% SDS at 42°C, as described in Molecular Cloning: A Laboratory Manual, Maniatis et al. Other useful procedures known in the art include solution hybridization, and dot and slot RNA hybridization. The amount of marker transcript present in a sample then is quantitated by measuring the radioactivity of hybridized fragments, using standard procedures known in the art.

Following a similar protocol, oligonucleotides also may be used to identify other sequences encoding members of the MT1 and MT2 protein families, for example, as described in the examples that follow. The methodology also may be used to identify genetic sequences associated with the protein coding sequences described herein, e.g., to identify noncoding sequences lying upstream or downstream of the protein coding sequence, and which may play a functional role in expression of these genes. Where new marker species are to be identified, degenerate sequences and/or sequences with preferred codon bias may be created, using the sequences of Seq. ID Nos. 1 or 3 as templates, and the general guidelines described in the art for incorporating degeneracies. (See, for example, Molecular Cloning: A Laboratory Manual, Maniatis, et al.)

C. Therapeutics

The proteins described herein are associated with the spindle apparatus during mitosis, and are present at elevated levels in malignant cells. Accordingly, without being limited to any particular theory, it is hypothesized that the proteins likely play a significant role in cell division, most likely a structurally related role. Accordingly, these proteins and their transcripts are good candidates as target molecules for a cancer chemotherapy.

C.1 Antisense Therapeutics

A particularly useful cancer therapeutic envisioned is an oligonucleotide complementary to part all of the marker transcript, capable of hybridizing specifically to the transcript and inhibiting translation of the mRNA when hybridized to the mRNA transcript. Antisense oligonucleotides have been used extensively to inhibit gene expression in normal and abnormal cells. See, for example, Stein et al. (1988) Cancer Res. 48:2659-2668, for a pertinent review of antisense theory and established protocols. Accordingly, the antisense nucleotides to MT1 and MT2 may be used as part of chemotherapy, alone or in combination with other therapies.

As described in Section I.B above, both oligoribonucleotide and oligodeoxyribonucleotide sequences will hybridize to an MRNA transcript and may be used to inhibit mRNA translation of the marker protein described herein. However, oligoribonucleotides generally are more susceptible to enzymatic attack by ribonucleases than deoxyribonucleotides. Hence, oligodeoxyribonucleotides are preferred for in vivo therapeutic use to inhibit mRNA translation in an individual.

Also, as described in Section I.B above, the therapeutically useful antisense oligonucleotides of the invention may be synthesized by any of the known chemical oligonucleotide synthesis methods well described in the art. Alternatively, a complementary sequence to part or all of the natural mRNA sequence

may be generated using standard recombinant DNA technology. For example, the DNA encoding the protein coding sequence may be inserted in reverse orientation downstream of a promoter capable of expressing the sequence such that the noncoding strand is transcribed.

Since the complete nucleotide sequence of the protein coding sequence as well as additional 5' and 3' untranslated sequences are known for both MT1 and MT2 (see, for example, Seq. ID Nos. 1 and 3 and Compton et al.), and/or can be determined with this disclosure, antisense oligonucleotides hybridizable with any portion of the mRNA transcripts to these proteins may be prepared using conventional oligonucleotide synthesis methods known to those skilled in the art.

Oligonucleotides complementary to and hybridizable with any portion of the MT1 and MT2 mRNA transcripts are, in principle, effective for inhibiting translation of the transcript as described herein. For example, as described in U.S. Pat. No. 5,098,890, issued March 24, 1992, the disclosure of which is incorporated herein by reference, oligonucleotides complementary to mRNA at or near the translation initiation codon site may be used to advantage to inhibit translation. Moreover, it has been suggested that sequences that are too distant in the 3' direction from the translation initiation site may be less effective in hybridizing the mRNA transcripts because of potential ribosomal "read-through", a phenomenon whereby the ribosome is postulated to unravel the antisense/sense duplex to permit translation of the message.

Representative antisense sequences for MT1 and MT2 transcripts are described in Seq. ID No. 5 (MT1) and Seq. ID No. 6 (MT2). The antisense sequences are complementary the sequence encoding the N-terminus of either the MT1 or MT2 marker protein, as well as part of the 5' untranslated sequences immediately upstream of the initiation codon. (See Section I.B, above for a detailed description of these sequences). As will be appreciated by those skilled in the art, antisense oligonucleotides complementary to other regions of the MT1 and/or MT2 transcripts are readily created using for example, the sequences presented in Seq. ID No. 1 and 3 as templates.

As described in Section I.B above, any length oligonucleotide may be utilized to hybridize to mRNA transcripts. However, very short sequences (e.g., less than 8-15 nucleotides) may bind with less specificity. Moreover, for in vivo use such short sequences may be particularly susceptible to enzymatic degradation. In addition, where oligonucleotides are to be provided directly to the cells, very long sequences may be less effective at inhibition because of decreased uptake by the target cell. Accordingly, where the oligonucleotide is to be provided directly to target cells, oligonucleotides having a length within the range of 8-50 nucleotides, preferably 15-30 nucleotides, are envisioned to be most advantageous.

An alternative means for providing antisense sequences to a target cell is as part of a gene therapy technique, e.g., as a DNA sequence, preferably part of a vector, and associated with a promoter capable of expressing the antisense sequence, preferably constitutively, inside the target cell. Recently,

Oeller et al. ((1992) Science 254:437-539, the disclosure of which is in corporated by reference) described the in vivo inhibition of the ACC synthase enzyme using a constitutively expressible DNA sequence encoding an antisense sequence to the full length ACC synthase transcript. Accordingly, where the antisense sequences are provided to a target cell indirectly, e.g., as part of an expressable gene sequence to be expressed within the cell, longer oligonucleotide sequences, including sequences complementary to substantially all the protein coding sequence, may be used to advantage.

Finally, also as described in Section I.B, above, the therapeutically usefully oligonucleotides envisioned include not only native oligomers composed of naturally occurring nucleotides, but also those comprising modified nucleotides to, for example, improve stability and lipid solubility and thereby enhance cellular uptake. For example, it is known that enhanced lipid solubility and/or resistance to nuclease digestion results by substituting a methyl group or sulfur atom for a phosphate oxygen in the internucleotide phosphodiester linkage. Phosphorothioates ("S-oligonucleotides" wherein a phosphate oxygen is replaced by a sulfur atom), in particular, are stable to nuclease cleavage, are soluble in lipids, and are preferred, particularly for direct oligonucleotide administration. S-oligonucleotides may be synthesized chemically by the known automated synthesis methods described in Section I.B, above.

Suitable oligonucleotide sequences for mRNA translation inhibition are readily evaluated by a standard in vitro assay using standard procedures described herein and well characterized in the art. An exemplary protocol is described below, but others are envisioned and may be used to advantage.

A candidate antisense sequence is prepared as provided herein, using standard chemical techniques. For example, an MT1 antisense sequence may be prepared having the sequence described by positions 285-315 of Sequence ID No. 5 using an Applied Biosystems automated DNA Synthesizer, and the oligonucleotide purified accordingly to manufacturer's instructions. The oligonucleotide then is provided to a suitable malignant cell line in culture, e.g., ME-180, under standard culture conditions, to be taken up by the proliferating cells.

Preferably, a range of doses is used to determine effective concentrations for inhibition as well as specificity of hybridization. For example, a dose range of 0-100 μg oligonucleotide/ml may be assayed. Further, the oligonucleotides may be provided to the cells in a single transfection, or as part of a series of transfections.

Antisense efficacy may be determined by assaying a change in cell proliferation over time following transfection, using standard cell counting methodology and/or by assaying for reduced expression of marker protein, e.g., by immunofluorescence, as described in Section I.A, above. Alternatively, the ability of

cells to take up and use thymidine is another standard means of assaying for cell division and maybe used here, e.g., using ^{3}H thymidine. Effective antisense inhibition should inhibit cell division sufficiently to reduce thymidine uptake, inhibit cell proliferation, and/or reduce detectable levels of marker proteins.

Useful concentration ranged are envisioned to vary according to the nature and extent of the neoplasm, the particular oligonucleotide utilized, the relative sensitivity of the neoplasm to the oligonucleotides, and other factors. Useful ranges for a given cell type and oligonucleotide may be determined by performing a standard dose range experiment as described here. Dose range experiments also may be performed to assess toxicity levels for normal and malignant cells. Concentrations from about 1 to 100 μg/ml per 10^5 cells may be employed to advantage.

For in vivo use, the antisense oligonucleotides may be combined with a pharmaceutical carrier, such as a suitable liquid vehicle or excipient and an optional auxiliary additive or additives. The liquid vehicles and excipients are conventional and commercially available. Illustrative thereof are distilled water, physiological saline, aqueous solutions of dextrose, and the like. For in vivo cancer therapies, the antisense sequences preferably are provided directly to the malignant cells, as by injection to the neoplasm locus. Alternatively, the oligonucletide may be administered systemically, provided that the antisense sequence is associated with means for directing the sequences to the target malignant cells.

In addition to administration with conventional carriers, the antisense oligonucleotides may be administered by a variety of specialized oligonucleotide delivery techniques. For example, oligonucleotides maybe encapsulated in liposomes, as described in Maniatis et al., Mannino et al. (1988) BioTechnology 6:682, and Felgner et al. (1989) Bethesda Res. Lab. Focus 11:21. Reconstituted virus envelopes also have been successfully used to deliver RNA and DNA to cells. (see, for example, Arad et. al., (1986) Biochem. Biophy. Acta. 859, 88-94.)

For therapeutic use in vivo, the antisense oligonucleotides are provided in a therapeutically effective amount, e.g., an amount sufficient to inhibit target protein expression in malignant cells. The actual dosage administered may take into account whether the nature of the treatment is prophylactic or therapeutic in nature, the age, weight, health and sex of the patient, the route of administration, the size and nature of the malignancy, as well as other factors. The daily dosage may range from about 0.01 to 1,000 mg per day. Greater or lesser amounts of oligonucleotide may be administered, as required. As will be appreciated by those skilled in the medical art, particularly the chemotherapeutic art, appropriate dose ranges for in vivo administration would be routine experimentation for a clinician. As a preliminary guideline, effective concentrations for in vitro inhibition of the target molecule may be determined first, as described above.

II.B PROTEIN INHIBITION

In another embodiment, the cancer marker protein itself may be used as a target molecule. For example, a binding protein designed to bind the marker protein essentially irreversibly can be provided to the malignant cells e.g., by association with a ligand specific for the cell and known to be absorbed by the cell. Means for targeting molecules to particular cells and cell types are well described in the chemotherapeutic art.

Binding proteins maybe obtained and tested as described in Section I.A above. For example, the binding portions of antibodies maybe used to advantage. Particularly useful are binding proteins identified with high affinity for the target protein, e.g., greater than about 10^9 M^{-1}. Alternatively, the DNA encoding the binding protein may be provided to the target cell as part of an expressable gene to be expressed within the cell following the procedures used for gene therapy protocols well described in the art. (see, for example, U.S. Patent No. 4,497,796, and Gene Transfer, Vijay R. Baichwal, ed., (1986). It is anticipated that the complexed INM protein will be disabled and can inhibit cell division thereby.

As described above for antisense nucleotides, for in vivo use, suitable binding proteins may be combined with a suitable pharmaceutical carrier, such as physiological saline or other useful carriers well characterized in the medical art. The parmaceutical compositions may be provided directly to malignant cells, e.g., by direct injection, or may be provided systemically, provided the binding protein is

associated with means for targeting the protein to target cells. Finally, suitable dose ranges and cell toxicity levels may be assessed using standard dose range experiments. Therapeutically effective concentrations may range from 0.1-1,000 mg per day. As described above, actual dosages administered may vary depending, for example, on the nature of the malignanacy, the age, weight and health of the individual, as well as other factors.

II. EXEMPLIFICATION

The following examples further describe the utility of MT1 and MT2 as markers for abnormal cell types, and how the genetic sequences encoding MT1 and MT2 proteins were isolated and characterized, including the current best mode for their cloning and characterization, without limiting the scope thereof. For example, INM protein expression in E. coli is described herein. However, other prokayrotic and eukaryotic cell expression systems also are contemplated for recombinant expression of the proteins described herein. Other useful hosts contemplated include Saccharomyces, the insect/baculovirus expression system, and mammalian cells such as xenogenic myeloma cells and the well-characterized chinese hamster ovary cell lines.

MT1

As demonstrated below, MT1 expression levels are enhanced significantly in a number of different malignant cell types, including malignant breast, colon, bladder, ovary, prostate and cervix cell types. Presented below are the results of a standard immunoassy (precision ± 5%), performed as described herein and in PCT publication WO93/03497 on nuclear matrix (NM) preparations made from normal and malignant human tissue extracts and which were prepared essentially as described therein (in 8M urea, 2% β-mercaptoethanol, 2% Nonidet P-40 (detergent).) The 302.47 antibody was raised against a NM preparation from CaSki, a cultured cervical tumor cell line (American Type Culture Collection, ATCC, Rockville, MD). MT1:2-8 was raised against the cloned MT1 protein. Both antibodies bind to epitopes on the protein encoded by Seq. ID No.1, as demonstrated using standard binding assays. As can be seen from the results presented below, MT1 is significantly elevated in malignant bladder tissue. Blotting experiments also indicate MT1 levels are elevated in other malignant tissues.

TABLE I

Sample	Antibody Combination	ng MT-1/ g tissue
normal bladder	302.47/MT1:2-8	13,500
bladder cancer	302.47/MT1:2-8	32,000

Cloning

The natural-sourced MT1 protein first was separated from human cervical tumor cells essentially following the procedure of Penman and Fey described in U.S. Pat. Nos. 4,882,268 and 4,885,236. Cells from the human

cervical tumor cell lines CaSki and ME180 (obtained from the American Tissue Culture Collection, ATCC, Rockville, MD) were grown to confluence and removed from flasks by trypsinization. Suspended cells were washed twice with phosphate buffered saline (PBS) and extracted with cytoskeletal buffer (CSK): 100 mM NaCl, 300 mM sucrose, 10 mM PIPES, 3 mM $MgCl_2$, 1 mM EGTA, 0.5% Triton X-100, 1.2 mM PMSF for 1 min at 4°C, followed by extraction in cold RSB (reticulocyte suspension buffer)/double detergent buffer: 100 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris, pH 7.4, 1% Tween 40, 0.5% deoxycholate, 1.2 mM PMSF. Alternatively, cells were extracted twice with the RBS/double detergent buffer. The two extraction protocols produced very similar preparations. The extracted cells were digested for 30 min at room temperature in digestion buffer: 50mM NaCl, 300 mM sucrose, 0.5% Triton X-100, 10 mM PIPES (pH 6.8), 3 mM $MgCl_2$, 1mM EGTA, 1.2 mM PMSF, containing 100 μg of both RNase A and DNase I. Chromatin was extracted from the digested nuclei by the addition of 2 M ammonium sulfate to a final concentration of 0.25 M. The extracted nuclear matrix-intermediate filament (NM-IF) scaffolds then were sedimented at 3700 x g for 15 min.

The resulting pellet then was resuspended in disassembly buffer: 8 M urea, 20 mM MES (pH 6.6), 1 mM EGTA, 1.2mM PMSF, 0.1 mM $MgCl_2$, 1% 2-mercaptoethanol, and the pellet sonicated and dialyzed overnight with 3 changes of 2000 volumes of assembly buffer: 0.15 M KCl, 25 mM imidazole (pH 7.1), 5 mM $MgCl_2$, 2 mM DTT, 0.125 mM EGTA, 0.2 mM PMSF. The dialysate then was centrifuged at 100k x g for 1 h and the NM proteins recovered from the supernatant. Alternatively, NM-IF scaffolds were extracted directly with E400 buffer:

0.4 M NaCl, 0.02 M Tris pH 7.5, 0.1 mM MCl_2, 0.5% 2-mercaptoethanol, 1.2 mM PMSF, for 30 min at 4°C, as described by von Kries et al. (1991) Cell 64:123-135. The intermediate filament-rich pellet then was removed after centrifugation for 90 min at 40K rpm in a Beckman 70.1 Ti rotor. The supernatant remaining is enriched in MT1 protein with little cytokeratin contamination.

MT1-specific antibodies were produced by standard procedures. Specifically, Balb/c by J mice (Jackson Laboratory, Bar Harbor, ME) were injected intraperitoneally with purified Caski NM protein every 2 weeks for a total of 16 weeks. The mice were injected with a single boost 4 days prior to sacrifice and removal of the spleen. Freund's complete adjuvant was used in the first injection, incomplete Freund's in the second injection; subsequent injections were made with saline. Spleen cells were fused with the SP2/O-Ag14 mouse myeloma line (ATCC, Rockville, MD) using the standard fusion methodologies well known in the art. Hybridomas producing antibodies that reacted with nuclear matrix proteins were cloned and grown as ascites. Antigen specificity was assessed both by immunoflourescence spectroscopy and Western blot analysis. The 302.47 antibody was used to screen an expression library as described below to isolate the MT1 gene.

The cDNA clones for MT1 were obtained from a Lambda ZAP expression library (Stratagene, La Jolla, CA). Library screening was carried out according to the manufacturer's instructions and using the MT1-speciifc antibody 302.47. Briefly, a single positive clone

containing a 2.45 kb insert was identified and subcloned into pBluescript II vectors (Stratagene, La Jolla, CA) opened at the EcoRI and XhoI cloning sites. The resulting plasmid, pMT1, was sequenced directly and further subcloned to produce the MT1 fusion protein (see below).

The cDNA sequences were obtained using the standard dideoxy method described in the art. Double stranded sequencing was done utilizing the pMT1 vector primed with appropriate primers according to manufacturer's instructions (Stratagene, La Jolla, CA). Internal sequences were obtained using synthetic primers, created based on the identified sequence.

The entire nucleotide sequence and predicted amino acid sequence for MT1 are shown in Seq. ID No. 1. The cDNA clone retains a polyadenylation signal a putative initiation codon, a continuous open reading frame and codon utilization consistent with a human gene. The predicted amino acid sequence of MT1 consists of 639 amino acids encoding a protein of 70.5 kD with a pI of 5.47. The primary structure, as predicted by the Chou-Fasman algorithm (Chou and Fasman, (1978) Adv. Enzymol. Relat. Areas Mol. Biol. 47:145-148), consists of 72% alpha helix of which 56% is extended helix.

The primary structure of MT1, represented in Fig. 1, contains 27 proline residues which generally occur in pairs or triplets throughout the molecule. The proline distribution within the sequence is illustrated in Fig. 1A, where diamonds represent the proline residues. Proline pairs and triplets are indicted by stacked diamonds. At the N terminus, a 40 amino acid stretch contains a cluster of 8 prolines

(residues 42-81, Seq. ID No. 1) that occur as pairs separated by 3 or fewer amino acids. A similar proline-rich region occurs in the C terminus of MT1 (residues 551-563) where 6 prolines occur in a 13 amino acid stretch. Both proline-rich regions likely lie on the protein surface, based on probability calculations determined by the technique of Emini et al. (1985) J. Virol. 55:836-839. The high proline density also may explain the anomalous apparent molecular weight of the protein as determined by SDS polyacrylamide gel electrophoresis. As described above, the predicted molecular weight for MT1, calculated from the amino acid sequence, is 70.1 kD. However, as described below, both the natural-sourced and recombinant protein migrate as a 90 kD protein on an SDS polyacrylamide gel. Alternatively, it is also possible that the molecular weight variation may result from some post-translational modification achievable in both prokaryotic and eukaryotic cells.

Between the two proline-rich termini, MT1 displays a sequence consistent with a region of extended alpha helix structure, indicated by the hatched structure in Fig. 1B. The extended helix is interrupted in 4 places by short helix-distorting amino acid stretches that usually include a pair of proline residues. A preliminary hypothesis as to the structure of MT1 based on these theoretical calculations is that the molecule consists of an extended rod that is bounded on either end by a globular, proline-rich domain.

Analysis of all available sequence databases indicates that MT1 has a novel sequence that bears no significant homology to any known protein. In

addition, the sequence appears to lack any known, identifiable DNA binding motif such as the leucine zipper motif.

The cloned MT1 DNA was used to perform standard Northern blot analysis of total and poly A+ RNA from ME180 cells, using standard procedures and 15 μg RNA. After blotting and hybridization with ^{32}P-labelled pMT1 DNA, a single mRNA band was detected in the poly A+ fraction. This band was not apparent in the total RNA lane after a 48 h exposure of the autoradiogram, indicating that the MT1 message is a low abundance species. Northern blot analysis indicates that the MT1 protein is translated from a single mRNA. Northern blot analysis also indicates that the MT1 RNA includes approximately 500 bp 5' of the protein-coding sequence presented in Seq. ID No. 1. This upstream sequence may represent one or more untranslated sequences and/or may encode additional protein coding sequences.

A fusion protein for MT1 was obtained using the insert from the pMT1 construct described above and in Seq. ID No. 1, and the pMAL expression system (New England Biolabs Inc., Beverly, MA). In this system the gene of interest (MT1) is cloned into the pMal-c vector (New England Biolabs Inc., Beverly, MA) and the vector transfected into E. coli and expressed to produce a fusion protein containing both the protein of interest and the maltose binding protein. The maltose binding protein allows the fusion protein to be selectively purified in the presence of maltose and can be subsequently cleaved by proteolytic clavage with Factor Xa to yield intact, recombinant MT1 protein. Here, MT1 cDNA was cloned into the pMAL-c vector such that the initiation AUG codon was directly continuous with the

5' terminus of the maltose binding protein. After proteolytic cleavage with factor Xa the resulting MT1 fusion protein retains the complete amino acid sequence encoded by the MT1 cDNA with no additional amino acids. All experimental details of the pMAL system were carried out according to the manufacturer's instructions.

As described above, both the natural-sourced and recombinantly produced protein have an electrophoretic mobility consistent with an apparent molecular weight of about 90kD on SDS-PAGE. In addition, the pI of both proteins is equivalent (5.4) and consistent with the predicted pI as calculated from the amino acid sequence. Peptide mapping of both proteins by cleavage at cysteine residues with 2-nitro-5-thiocyanobenzoic acid (NTCB), following the method of Leto and Marchesi (1984) J. Biol. Chem. 259:4603-4049, yields equivalent peptide fragments which share the same MT1 cross reactivity by Western blot analysis. Moreover, the number and size of the peptide fragments produced are consistent with those predicted from the proposed MT1 amino acid sequence.

MT2

Like MT1, MT2 expression levels are enhanced significantly in a variety of malignant cell types, as determined both by serum assays and tissue culture supernatant assays. In the assays described below, the antibodies used were raised against two different cervical tumor cell line NM preparations (ME-180 and CaSKi, ATTC, Rockville, MD.) The 100-series antibodies are those raised against the ME-180 immunogen; the 300-

series antibodies are those raised against CaSKi-NM immunogen. Of the antibodies described below, 107.7 and 307.33 have been determined to bind specifically with the MT2 protein, and 302-18, 302-22 and 302-29 cross react with a protein closely associated with MT2 and which co-isolates with it.

Dose response evaluation results of two antibody combinations are shown in Table II, below, using ME-180 cell culture supernatant as the antigen source. Each assay shows dose dependent detection of antigen in the tissue culture supernatant, demonstrating the ability of the assay to quantitate soluble interior nuclear matrix protein released from dying cells.

Table II

Antibody 107-7 solid phase, 302-29 soluble antibody, ME-180 supernatant

Concentration of supernatant	Mean OD	SD
2:1	0.501	0.013
undiluted	0.274	0.018
1:2	0.127	0.006
1:4	0.067	0.006
1:8	0.035	0.009
1:16	0.021	0.007
No Sup	0.000	

Antibody 107-7 solid phase, 307-33 soluble antibody, ME-180 supernatant.

Concentration of supernatant	Mean OD	SD
3:1	0.906	0.009
3:2	0.456	0.011
3:4	0.216	0.007
3:8	0.099	0.005
3:16	0.052	0.002
3:32	0.031	0.005
No Sup		

Next, interior nuclear matrix protein quantification was tested in supernatant from a variety of dying tumor tissues. Here, tumor and normal tissues were allowed to die in media by serum deprivation. Specifically, cell lines were grown to confluency in tissue culture flasks by standard culturing techniques. The media then was replaced with serum-free media and the cells placed in a 37°C incubator with 5% CO_2 for 7 to 14 days. At the end of the incubation the media was collected and centrifuged at 14,000xg to remove cellular debris. Supernatants were assayed in various configurations of sandwich assays.

The results are shown in Fig. 3, where all values are in units/gm, using ME-180 antigen as standard. As can be seen from Fig. 3, MT2 antigen is released from each of the dying tissues and the increased cell death in tumor tissue is reflected in a higher MT2 average antigen value quantitated in cancer tissue versus normal tissue.

Figure 4 shows the results of an analogous experiment performed using serum samples from cancer patients and normal blood donors. Here tissue is prepared as follows. Tissue is removed from a donor, flash frozen in liquid nitrogen within 10min to 4hrs after removal and stored at -70°c until required. When ready to be used, the tissue is chopped into 0.1 to 0.3 cm cubes as it thaws using aseptic techniques in a laminar flow hood and placed in a T150 flask containing serum free media containing Fungizone and gentamycin. In general, 2-4g of tissue are used per 100ml media in the T150 flask. The flask containing the tissue then is incubated for 4-7 days at 37°c with 5% CO_2. After incubation the media is collected from the flasks,

centrifuged at 14,000xg for 20min. As for Fig. 3, ME-180 cell antigen was the standard. Results are reported in units/ml. A control experiment diluting supernatant antigen into serum and then quantitating the protein in solution indicates that serum has little or no effect on the assay. As can be seen in the results presented in Fig. 4, like the results shown in Fig. 3, serum samples from cancer patients reflect a higher rate of cell death as indicated by the quantifiably higher levels of MT2 antigen detected in these samples compared with those detected in the normal blood serum samples.

Cloning

Following the same general procedure as for MT1, a composition selectively enriched for MT2 was obtained from ME-180 cells (cervical carcinoma cells, from ATCC, Rockville, MD), and MT2-specific antibodies prepared. The 107.7 antibody was used to obtained a partial cDNA clone for MT2, by screening a lambda ZAP expression library, as for MT-1. The partial clone retrieved then was subcloned into a pBluescript II vector (pMT2) and the MT2 cDNA sequenced using standard techniques. The sequenced DNA, which corresponds to residues 1366 to 2865 of Seq. ID No. 3, then was analyzed to determine the reading frame and encoded amino acid sequence. The complete coding sequence subsequently was determined and is presented in Seq. ID No. 3. (Compton et al. (1992) J. Cell Biol. 116: 1395-1408). The nucleotide sequence and predicted amino acid sequence for MT2 are described in Seq. ID No. 3.

The primary structure of MT2 is represented schematically in Fig. 2: The protein appears to comprise at least 6 helical regions separated by proline pairs, (See Fig. 4A and B.) The primary structure may allow the protein to form a coiled-coil structure in solution. As for Fig. 3, prolines are indicated by diamonds and helices by hatched boxes. In addition, both the N and C termini of MT2 appear to fold as globular domains (Compton et al. (1992) J. Cell Biol. 116: 1395-1408.)

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Matritech, Inc.
(B) STREET: 763D Concord Ave
(C) CITY: Cambridge
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 02138
(G) TELELPHONE: 1-617-661-6660
(H) TELEFAX: 1-617-661-8522
(I) TELEX:

(ii) TITLE OF INVENTION: NOVEL MALIGNANT CELL TYPE MARKERS OF THE INTERIOR NUCLEAR MATRIX

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: TESTA HURWITZ & THIBEAULT
(B) STREET: 53 STATE STREET
(C) CITY: BOSTON
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: PITCHER ESQ, EDMUND R
(B) REGISTRATION NUMBER: 27,829
(C) REFERENCE/DOCKET NUMBER: MTP-013

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617/248-7000
(B) TELEFAX: 617/248-7100

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: CERVIX TUMOR

```
GAGATGGTTC TTGGTCCTGC AGCTTATAAT GTTCCATTGC CAAAGAAATC GATTCAGTCG      60

GGTCCACTAA AAATCTCTAG TGTATCAGAA GTA ATG AAA GAA TCT AAA CAG CCT      114
                                     Met Lys Glu Ser Lys Gln Pro
                                       1               5

GCC TCA CAA CTC CAA AAA CAA AAG GGA GAT ACT CCA GCT TCA GCA ACA      162
Ala Ser Gln Leu Gln Lys Gln Lys Gly Asp Thr Pro Ala Ser Ala Thr
         10                  15                  20

GCA CCT ACA GAA GCG GCT CAA ATT ATT TCT GCA GCA GGT GAT ACC CTG      210
Ala Pro Thr Glu Ala Ala Gln Ile Ile Ser Ala Ala Gly Asp Thr Leu
     25                  30                  35

TCG GTC CCA GCC CCT GCA GTT CAG CCT GAG GAA TCT TTA AAA ACT GAT      258
Ser Val Pro Ala Pro Ala Val Gln Pro Glu Glu Ser Leu Lys Thr Asp
 40                  45                  50                  55

CAC CCT GAA ATT GGT GAA GGA AAA CCC ACA CCT GCA CTT TCA GAA GCA      306
His Pro Glu Ile Gly Glu Gly Lys Pro Thr Pro Ala Leu Ser Glu Ala
                 60                  65                  70

TCC TCA TCT TCT ATA AGG GAG CGA CCA CCT GAA GAA GTT GCA GCT CGC      354
Ser Ser Ser Ser Ile Arg Glu Arg Pro Pro Glu Glu Val Ala Ala Arg
         75                  80                  85

CTT GCA CAA CAG GAA AAA CAA GAA CAA GTT AAA ATT GAG TCT CTA GCC      402
Leu Ala Gln Gln Glu Lys Gln Glu Gln Val Lys Ile Glu Ser Leu Ala
         90                  95                  100

AAG AGC TTA GAA GAT GCT CTG AGG CAA ACT GCA AGT GTC ACT CTG CAG      450
Lys Ser Leu Glu Asp Ala Leu Arg Gln Thr Ala Ser Val Thr Leu Gln
     105                 110                 115

GCT ATT GCA GCT CAG AAT GCT GCG GTC CAG GCT GTC AAT GCA CAC TCC      498
Ala Ile Ala Ala Gln Asn Ala Ala Val Gln Ala Val Asn Ala His Ser
120                 125                 130                 135
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AAC ATA TTG AAA GCC GCC ATG GAC AAT TCT GAG ATT GCA GGC GAG AAG      546
Asn Ile Leu Lys Ala Ala Met Asp Asn Ser Glu Ile Ala Gly Glu Lys
                140                 145                 150

AAA TCT GCT CAG TGG CGC ACA GTG GAG GGT GCA TTG AAG GAA CGC AGA      594
Lys Ser Ala Gln Trp Arg Thr Val Glu Gly Ala Leu Lys Glu Arg Arg
            155                 160                 165

AAG GCA GTA GAT GAA GCT GCC GAT GCC CTT CTC AAA GCC AAA GAA GAG      642
Lys Ala Val Asp Glu Ala Ala Asp Ala Leu Leu Lys Ala Lys Glu Glu
        170                 175                 180

TTA GAG AAG ATG AAA AGT GTG ATT GAA AAT GCA AAG AAA AAA GAG GTT      690
Leu Glu Lys Met Lys Ser Val Ile Glu Asn Ala Lys Lys Lys Glu Val
    185                 190                 195

GCT GGG GCC AAG CCT CAT ATA ACT GCT GCA GAG GGT AAA CTT CAC AAC      738
Ala Gly Ala Lys Pro His Ile Thr Ala Ala Glu Gly Lys Leu His Asn
200                 205                 210                 215

ATG ATA GTT GAT CTG GAT AAT GTG GTC AAA AAG GTC CAA GCA GCT CAG      786
Met Ile Val Asp Leu Asp Asn Val Val Lys Lys Val Gln Ala Ala Gln
                220                 225                 230

TCT GAG GCT AAG GTT GTA TCT CAG TAT CAT GAG CTG GTG GTC CAA GCT      834
Ser Glu Ala Lys Val Val Ser Gln Tyr His Glu Leu Val Val Gln Ala
            235                 240                 245

CGG GAT GAC TTT AAA CGA GAG CTG GAC AGT ATT ACT CCA GAA GTC CTT      882
Arg Asp Asp Phe Lys Arg Glu Leu Asp Ser Ile Thr Pro Glu Val Leu
        250                 255                 260

CCT GGG TGG AAA GGA ATG AGT GTT TCA GAC TTA GCT GAC AAG CTC TCT      930
Pro Gly Trp Lys Gly Met Ser Val Ser Asp Leu Ala Asp Lys Leu Ser
    265                 270                 275

ACT GAT GAT CTG AAC TCC CTC ATT GCT CAT GCA CAT CGT CGT ATT GAT      978
Thr Asp Asp Leu Asn Ser Leu Ile Ala His Ala His Arg Arg Ile Asp
280                 285                 290                 295

CAG CTG AAC AGA GAG CTG GCA GAA CAG AAG GCC ACC GAA AAG CAG CAC     1026
Gln Leu Asn Arg Glu Leu Ala Glu Gln Lys Ala Thr Glu Lys Gln His
                300                 305                 310

ATC ACG TTA GCC TTG GAG AAA CAA AAG CTG GAA GAA AAG CGG GCA TTT     1074
Ile Thr Leu Ala Leu Glu Lys Gln Lys Leu Glu Glu Lys Arg Ala Phe
            315                 320                 325

GAC TCT GCA GTA GCA AAA GCA TTA GAA CAT CAC AGA AGT GAA ATA CAG     1122
Asp Ser Ala Val Ala Lys Ala Leu Glu His His Arg Ser Glu Ile Gln
        330                 335                 340
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GCT GAA CAG GAC AGA AAG ATA GAA GAA GTC AGA GAT GCC ATG GAA AAT      1170
Ala Glu Gln Asp Arg Lys Ile Glu Glu Val Arg Asp Ala Met Glu Asn
    345                 350                 355

GAA ATG AGA ACC CCT TCG CCG ACA GCA GCT GCC CAC ACT GAT CAC TTG      1218
Glu Met Arg Thr Pro Ser Pro Thr Ala Ala Ala His Thr Asp His Leu
360                 365                 370                 375

CGA GAT GTC CTT AGG GTA CAA GAA CAG GAA TTG AAG TCT GAA TTT GAG      1266
Arg Asp Val Leu Arg Val Gln Glu Gln Glu Leu Lys Ser Glu Phe Glu
                380                 385                 390

CAG AAC CTG TCT GAG AAA CTC TCT GAA CAA GAA TTA CAA TTT CGT CGT      1314
Gln Asn Leu Ser Glu Lys Leu Ser Glu Gln Glu Leu Gln Phe Arg Arg
            395                 400                 405

CTC AGT CAA GAG CAA GTT GAC AAC TTT ACT CTG GAT ATA AAT ACT GCC      1362
Leu Ser Gln Glu Gln Val Asp Asn Phe Thr Leu Asp Ile Asn Thr Ala
        410                 415                 420

TAT GCC AGA CTC AGA GGA ATC GAA CAG GCT GTT CAG AGC CAT GCA GTT      1410
Tyr Ala Arg Leu Arg Gly Ile Glu Gln Ala Val Gln Ser His Ala Val
    425                 430                 435

GCT GAA GAG GAA GCC AGA AAA GCC CAC CAA CTC TGG CTT TCA GTG GAG      1458
Ala Glu Glu Glu Ala Arg Lys Ala His Gln Leu Trp Leu Ser Val Glu
440                 445                 450                 455

GCA TTA AAG TAC AGC ATG AAG ACC TCA TCT GCA GAA ACA CCT ACT ATC      1506
Ala Leu Lys Tyr Ser Met Lys Thr Ser Ser Ala Glu Thr Pro Thr Ile
                460                 465                 470

CCG CTG GGT AGT GCG GTT GAG GCC ATC AAA GCC AAC TGT TCT GAT AAT      1554
Pro Leu Gly Ser Ala Val Glu Ala Ile Lys Ala Asn Cys Ser Asp Asn
            475                 480                 485

GAA TTC ACC CAA GCT TTA ACC GCA GCT ATC CCT CCA GAG TCC CTG ACC      1602
Glu Phe Thr Gln Ala Leu Thr Ala Ala Ile Pro Pro Glu Ser Leu Thr
        490                 495                 500

CGT GGG GTG TAC AGT GAA GAG ACC CTT AGA GCC CGT TTC TAT GCT GTT      1650
Arg Gly Val Tyr Ser Glu Glu Thr Leu Arg Ala Arg Phe Tyr Ala Val
    505                 510                 515

CAA AAA CTG GCC CGA AGG GTA GCA ATG ATT GAT GAA ACC AGA AAT AGC      1698
Gln Lys Leu Ala Arg Arg Val Ala Met Ile Asp Glu Thr Arg Asn Ser
520                 525                 530                 535

TTG TAC CAG TAC TTC CTC TCC TAC CTA CAG TCC CTG CTC CTA TTC CCA      1746
Leu Tyr Gln Tyr Phe Leu Ser Tyr Leu Gln Ser Leu Leu Leu Phe Pro
                540                 545                 550
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CCT CAG CAA CTG AAG CCG CCC CCA GAG CTC TGC CCT GAG GAT ATA AAC      1794
Pro Gln Gln Leu Lys Pro Pro Pro Glu Leu Cys Pro Glu Asp Ile Asn
            555                 560                 565

ACA TTT AAA TTA CTG TCA TAT GCT TCC TAT TGC ATT GAG CAT GGT GAT      1842
Thr Phe Lys Leu Leu Ser Tyr Ala Ser Tyr Cys Ile Glu His Gly Asp
        570                 575                 580

CTG GAG CTA GCA GCA AAG TTT GTC AAT CAG CTG AAG GGG GAA TCC AGA      1890
Leu Glu Leu Ala Ala Lys Phe Val Asn Gln Leu Lys Gly Glu Ser Arg
    585                 590                 595

CGA GTG GCA CAG GAC TGG CTG AAG GAA GCC CGA ATG ACC CTA GAA ACG      1938
Arg Val Ala Gln Asp Trp Leu Lys Glu Ala Arg Met Thr Leu Glu Thr
600                 605                 610                 615

AAA CAG ATA GTG GAA ATC CTG ACA GCA TAT GCC AGC GCC GTA GGA ATA      1986
Lys Gln Ile Val Glu Ile Leu Thr Ala Tyr Ala Ser Ala Val Gly Ile
                620                 625                 630

GGA ACC ACT CAG GTG CAG CCA GAG TGAGGTTTAG GAAGATTTTC ATAAAGTCAT     2040
Gly Thr Thr Gln Val Gln Pro Glu
            635

ATTTCATGTC AAAGGAAATC AGCAGTGATA GATGAAGGGT TCGCAGCGAG AGTCCCGGAC    2100

TTGTCTAGAA ATGAGCAGGT TTACAAGTAC TGTTCTAAAT GTTAACACCT GTTGCATTTA    2160

TATTCTTTCC ATTTGCTATC ATGTCAGTGA ACGCCAGGAG TGCTTTCTTT GCAACTTGTG    2220

TAACATTTTC TGTTTTTTCA GGTTTTACTG ATGAGGCTTG TGAGGCCAAT CAAAATAATG    2280

TTTGTGATCT CTACTACTGT TGATTTTGCC CTCGGAGCAA ACTGAATAAA GCAACAAGAT    2340

GAAAAAAAAA AAAAAAAAAA                                               2360
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 639 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Lys Glu Ser Lys Gln Pro Ala Ser Gln Leu Gln Lys Gln Lys Gly
 1               5                  10                  15

Asp Thr Pro Ala Ser Ala Thr Ala Pro Thr Glu Ala Ala Gln Ile Ile
            20                  25                  30
```

```
Ser Ala Ala Gly Asp Thr Leu Ser Val Pro Ala Pro Ala Val Gln Pro
        35                  40                  45

Glu Glu Ser Leu Lys Thr Asp His Pro Glu Ile Gly Glu Gly Lys Pro
    50                  55                  60

Thr Pro Ala Leu Ser Glu Ala Ser Ser Ser Ser Ile Arg Glu Arg Pro
65                  70                  75                  80

Pro Glu Glu Val Ala Ala Arg Leu Ala Gln Gln Glu Lys Gln Glu Gln
                85                  90                  95

Val Lys Ile Glu Ser Leu Ala Lys Ser Leu Glu Asp Ala Leu Arg Gln
            100                 105                 110

Thr Ala Ser Val Thr Leu Gln Ala Ile Ala Ala Gln Asn Ala Ala Val
        115                 120                 125

Gln Ala Val Asn Ala His Ser Asn Ile Leu Lys Ala Ala Met Asp Asn
    130                 135                 140

Ser Glu Ile Ala Gly Glu Lys Lys Ser Ala Gln Trp Arg Thr Val Glu
145                 150                 155                 160

Gly Ala Leu Lys Glu Arg Arg Lys Ala Val Asp Glu Ala Ala Asp Ala
                165                 170                 175

Leu Leu Lys Ala Lys Glu Glu Leu Glu Lys Met Lys Ser Val Ile Glu
            180                 185                 190

Asn Ala Lys Lys Lys Glu Val Ala Gly Ala Lys Pro His Ile Thr Ala
        195                 200                 205

Ala Glu Gly Lys Leu His Asn Met Ile Val Asp Leu Asp Asn Val Val
    210                 215                 220

Lys Lys Val Gln Ala Ala Gln Ser Glu Ala Lys Val Val Ser Gln Tyr
225                 230                 235                 240

His Glu Leu Val Val Gln Ala Arg Asp Asp Phe Lys Arg Glu Leu Asp
                245                 250                 255

Ser Ile Thr Pro Glu Val Leu Pro Gly Trp Lys Gly Met Ser Val Ser
            260                 265                 270

Asp Leu Ala Asp Lys Leu Ser Thr Asp Asp Leu Asn Ser Leu Ile Ala
        275                 280                 285

His Ala His Arg Arg Ile Asp Gln Leu Asn Arg Glu Leu Ala Glu Gln
    290                 295                 300
```

Lys Ala Thr Glu Lys Gln His Ile Thr Leu Ala Leu Glu Lys Gln Lys
305 310 315 320

Leu Glu Glu Lys Arg Ala Phe Asp Ser Ala Val Ala Lys Ala Leu Glu
325 330 335

His His Arg Ser Glu Ile Gln Ala Glu Gln Asp Arg Lys Ile Glu Glu
340 345 350

Val Arg Asp Ala Met Glu Asn Glu Met Arg Thr Pro Ser Pro Thr Ala
355 360 365

Ala Ala His Thr Asp His Leu Arg Asp Val Leu Arg Val Gln Glu Gln
370 375 380

Glu Leu Lys Ser Glu Phe Glu Gln Asn Leu Ser Glu Lys Leu Ser Glu
385 390 395 400

Gln Glu Leu Gln Phe Arg Arg Leu Ser Gln Glu Gln Val Asp Asn Phe
405 410 415

Thr Leu Asp Ile Asn Thr Ala Tyr Ala Arg Leu Arg Gly Ile Glu Gln
420 425 430

Ala Val Gln Ser His Ala Val Ala Glu Glu Glu Ala Arg Lys Ala His
435 440 445

Gln Leu Trp Leu Ser Val Glu Ala Leu Lys Tyr Ser Met Lys Thr Ser
450 455 460

Ser Ala Glu Thr Pro Thr Ile Pro Leu Gly Ser Ala Val Glu Ala Ile
465 470 475 480

Lys Ala Asn Cys Ser Asp Asn Glu Phe Thr Gln Ala Leu Thr Ala Ala
485 490 495

Ile Pro Pro Glu Ser Leu Thr Arg Gly Val Tyr Ser Glu Glu Thr Leu
500 505 510

Arg Ala Arg Phe Tyr Ala Val Gln Lys Leu Ala Arg Arg Val Ala Met
515 520 525

Ile Asp Glu Thr Arg Asn Ser Leu Tyr Gln Tyr Phe Leu Ser Tyr Leu
530 535 540

Gln Ser Leu Leu Leu Phe Pro Pro Gln Gln Leu Lys Pro Pro Pro Glu
545 550 555 560

Leu Cys Pro Glu Asp Ile Asn Thr Phe Lys Leu Leu Ser Tyr Ala Ser
565 570 575

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Tyr Cys Ile Glu His Gly Asp Leu Glu Leu Ala Ala Lys Phe Val Asn
          580                 585                 590

Gln Leu Lys Gly Glu Ser Arg Arg Val Ala Gln Asp Trp Leu Lys Glu
        595                 600                 605

Ala Arg Met Thr Leu Glu Thr Lys Gln Ile Val Glu Ile Leu Thr Ala
    610                 615                 620

Tyr Ala Ser Ala Val Gly Ile Gly Thr Thr Gln Val Gln Pro Glu
625                 630                 635
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6306 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..6306

(x) PUBLICATION INFORMATION:
(A) AUTHORS: COMPTON, DUANE A; SZILAK, ILYA; CLEVELAND, DON W.
(B) PRIMARY STRUCTURE OF NUMA...
(C) JOURNAL: Journal of Cell Biology
(D) VOLUME: 116
(E) RELEVANT RESIDUES IN SEQ ID NO:3: FROM 1 TO 6306
(F) PAGES: 1395-1408
(G) DATE: MAR - 1992

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG ACA CTC CAC GCC ACC CGG GGG GCT GCA CTC CTC TCT TGG GTG AAC        48
Met Thr Leu His Ala Thr Arg Gly Ala Ala Leu Leu Ser Trp Val Asn
  1               5                  10                  15

AGT CTA CAC GTG GCT GAC CCT GTG GAG GCT GTG CTG CAG CTC CAG GAC        96
Ser Leu His Val Ala Asp Pro Val Glu Ala Val Leu Gln Leu Gln Asp
            20                  25                  30

TGC AGC ATC TTC ATC AAG ATC ATT GAC AGA ATC CAT GGC ACT GAA GAG       144
Cys Ser Ile Phe Ile Lys Ile Ile Asp Arg Ile His Gly Thr Glu Glu
          35                  40                  45
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GGA CAG CAA ATC TTG AAG CAG CCG GTG TCA GAG AGA CTG GAC TTT GTG        192
Gly Gln Gln Ile Leu Lys Gln Pro Val Ser Glu Arg Leu Asp Phe Val
     50                  55                  60

TGC AGT TTT CTG CAG AAA AAT CGA AAA CAT CCC TCT TCC CCA GAA TGC        240
Cys Ser Phe Leu Gln Lys Asn Arg Lys His Pro Ser Ser Pro Glu Cys
 65                  70                  75                  80

CTG GTA TCT GCA CAG AAG GTG CTA GAG GGA TCA GAG CTG GAA CTG GCG        288
Leu Val Ser Ala Gln Lys Val Leu Glu Gly Ser Glu Leu Glu Leu Ala
                 85                  90                  95

AAG ATG ACC ATG CTG CTC TTA TAC CAC TCT ACC ATG AGC TCC AAA AGT        336
Lys Met Thr Met Leu Leu Leu Tyr His Ser Thr Met Ser Ser Lys Ser
            100                 105                 110

CCC AGG GAC TGG GAA CAG TTT GAA TAT AAA ATT CAG GCT GAG TTG GCT        384
Pro Arg Asp Trp Glu Gln Phe Glu Tyr Lys Ile Gln Ala Glu Leu Ala
        115                 120                 125

GTC ATT CTT AAA TTT GTG CTG GAC CAT GAG GAC GGG CTA AAC CTT AAT        432
Val Ile Leu Lys Phe Val Leu Asp His Glu Asp Gly Leu Asn Leu Asn
    130                 135                 140

GAG GAC CTA GAG AAC TTC CTA CAG AAA GCT CCT GTG CCT TCT ACC TGT        480
Glu Asp Leu Glu Asn Phe Leu Gln Lys Ala Pro Val Pro Ser Thr Cys
145                 150                 155                 160

TCT AGC ACA TTC CCT GAA GAG CTC TCC CCA CCT AGC CAC CAG GCC AAG        528
Ser Ser Thr Phe Pro Glu Glu Leu Ser Pro Pro Ser His Gln Ala Lys
                165                 170                 175

AGG GAG ATT CGC TTC CTA GAG CTA CAG AAG GTT GCC TCC TCT TCC AGT        576
Arg Glu Ile Arg Phe Leu Glu Leu Gln Lys Val Ala Ser Ser Ser Ser
            180                 185                 190

GGG AAC AAC TTT CTC TCA GGT TCT CCA GCT TCT CCC ATG GGT GAT ATC        624
Gly Asn Asn Phe Leu Ser Gly Ser Pro Ala Ser Pro Met Gly Asp Ile
        195                 200                 205

CTG CAG ACC CCA CAG TTC CAG ATG AGA CGG CTG AAG AAG CAG CTT GCT        672
Leu Gln Thr Pro Gln Phe Gln Met Arg Arg Leu Lys Lys Gln Leu Ala
    210                 215                 220

GAT GAG AGA AGT AAT AGG GAT GAG CTG GAG CTG GAG CTA GCT GAG AAC        720
Asp Glu Arg Ser Asn Arg Asp Glu Leu Glu Leu Glu Leu Ala Glu Asn
225                 230                 235                 240

CGC AAG CTC CTC ACC GAG AAG GAT GCA CAG ATA GCC ATG ATG CAG CAG        768
Arg Lys Leu Leu Thr Glu Lys Asp Ala Gln Ile Ala Met Met Gln Gln
                245                 250                 255
```

```
CGC ATT GAC CGC CTA GCC CTG CTG AAT GAG AAG CAG GCG GCC AGC CCA      816
Arg Ile Asp Arg Leu Ala Leu Leu Asn Glu Lys Gln Ala Ala Ser Pro
            260                 265                 270

CTG GAG CCC AAG GAG CTT GAG GAG CTG CGT GAC AAG AAT GAG AGC CTT      864
Leu Glu Pro Lys Glu Leu Glu Glu Leu Arg Asp Lys Asn Glu Ser Leu
        275                 280                 285

ACC ATG CGG CTG CAT GAA ACC CTG AAG CAG TGC CAG GAC CTG AAG ACA      912
Thr Met Arg Leu His Glu Thr Leu Lys Gln Cys Gln Asp Leu Lys Thr
    290                 295                 300

GAG AAG AGC CAG ATG GAT CGC AAA ATC AAC CAG CTT TCG GAG GAG AAT      960
Glu Lys Ser Gln Met Asp Arg Lys Ile Asn Gln Leu Ser Glu Glu Asn
305                 310                 315                 320

GGA GAC CTT TCC TTT AAG CTG CGG GAG TTT GCC AGT CAT CTG CAG CAG     1008
Gly Asp Leu Ser Phe Lys Leu Arg Glu Phe Ala Ser His Leu Gln Gln
                325                 330                 335

CTA CAG GAT GCC CTC AAT GAG CTG ACG GAG GAG CAC AGC AAG GCC ACT     1056
Leu Gln Asp Ala Leu Asn Glu Leu Thr Glu Glu His Ser Lys Ala Thr
            340                 345                 350

CAG GAG TGG CTA GAG AAG CAG GCC CAG CTG GAG AAG GAG CTC AGC GCA     1104
Gln Glu Trp Leu Glu Lys Gln Ala Gln Leu Glu Lys Glu Leu Ser Ala
        355                 360                 365

GCC CTG CAG GAC AAG AAA TGC CTT GAA GAG AAG AAC GAA ATC CTT CAG     1152
Ala Leu Gln Asp Lys Lys Cys Leu Glu Glu Lys Asn Glu Ile Leu Gln
    370                 375                 380

GGA AAA CTT TCA CAG CTG GAA GAA CAC TTG TCC CAG CTG CAG GAT AAC     1200
Gly Lys Leu Ser Gln Leu Glu Glu His Leu Ser Gln Leu Gln Asp Asn
385                 390                 395                 400

CCA CCC CAG GAG AAG GGC GAG GTG CTG GGT GAT GTC TTG CAG CTG GAA     1248
Pro Pro Gln Glu Lys Gly Glu Val Leu Gly Asp Val Leu Gln Leu Glu
                405                 410                 415

ACC TTG AAG CAA GAG GCA GCC ACT CTT GCT GCA AAC AAC ACA CAG CTC     1296
Thr Leu Lys Gln Glu Ala Ala Thr Leu Ala Ala Asn Asn Thr Gln Leu
            420                 425                 430

CAA GCC AGG GTA GAG ATG CTG GAG ACT GAG CGA GGC CAG CAG GAA GCC     1344
Gln Ala Arg Val Glu Met Leu Glu Thr Glu Arg Gly Gln Gln Glu Ala
        435                 440                 445

AAG CTG CTT GCT GAG CGG GGC CAC TTC GAA GAA GAA AAG CAG CAG CTG     1392
Lys Leu Leu Ala Glu Arg Gly His Phe Glu Glu Glu Lys Gln Gln Leu
    450                 455                 460
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TCT AGC CTG ATC ACT GAC CTG CAG AGC TCC ATC TCC AAC CTC AGC CAG      1440
Ser Ser Leu Ile Thr Asp Leu Gln Ser Ser Ile Ser Asn Leu Ser Gln
465                 470                 475                 480

GCC AAG GAA GAG CTG GAG CAG GCC TCC CAG GCT CAT GGG GCC CGG TTG      1488
Ala Lys Glu Glu Leu Glu Gln Ala Ser Gln Ala His Gly Ala Arg Leu
                485                 490                 495

ACT GCC CAG GTG GCC TCT CTG ACC TCT GAG CTC ACC ACA CTC AAT GCC      1536
Thr Ala Gln Val Ala Ser Leu Thr Ser Glu Leu Thr Thr Leu Asn Ala
            500                 505                 510

ACC ATC CAG CAA CAG GAT CAA GAA CTG GCT GGC CTG AAG CAG CAG GCC      1584
Thr Ile Gln Gln Gln Asp Gln Glu Leu Ala Gly Leu Lys Gln Gln Ala
        515                 520                 525

AAA GAG AAG CAG GCC CAG CTA GCA CAG ACC CTC CAA CAG CAA GAA CAG      1632
Lys Glu Lys Gln Ala Gln Leu Ala Gln Thr Leu Gln Gln Gln Glu Gln
    530                 535                 540

GCC TCC CAG GGC CTC CGC CAC CAG GTG GAG CAG CTA AGC AGT AGC CTG      1680
Ala Ser Gln Gly Leu Arg His Gln Val Glu Gln Leu Ser Ser Ser Leu
545                 550                 555                 560

AAG CAG AAG GAG CAG CAG TTG AAG GAG GTA GCG GAG AAG CAG GAG GCA      1728
Lys Gln Lys Glu Gln Gln Leu Lys Glu Val Ala Glu Lys Gln Glu Ala
                565                 570                 575

ACT AGG CAG GAC CAT GCC CAG CAA CTG GCC ACT GCT GCA GAG GAG CGA      1776
Thr Arg Gln Asp His Ala Gln Gln Leu Ala Thr Ala Ala Glu Glu Arg
            580                 585                 590

GAG GCC TCC TTA AGG GAG CGG GAT GCG GCT CTC AAG CAG CTG GAG GCA      1824
Glu Ala Ser Leu Arg Glu Arg Asp Ala Ala Leu Lys Gln Leu Glu Ala
        595                 600                 605

CTG GAG AAG GAG AAG GCT GCC AAG CTG GAG ATT CTG CAG CAG CAA CTT      1872
Leu Glu Lys Glu Lys Ala Ala Lys Leu Glu Ile Leu Gln Gln Gln Leu
    610                 615                 620

CAG GTG GCT AAT GAA GCC CGG GAC AGT GCC CAG ACC TCA GTG ACA CAG      1920
Gln Val Ala Asn Glu Ala Arg Asp Ser Ala Gln Thr Ser Val Thr Gln
625                 630                 635                 640

GCC CAG CGG GAG AAG GCA GAG CTG AGC CGG AAG GTG GAG GAA CTC CAG      1968
Ala Gln Arg Glu Lys Ala Glu Leu Ser Arg Lys Val Glu Glu Leu Gln
                645                 650                 655

GCC TGT GTT GAG ACA GCC CGC CAG GAA CAG CAT GAG GCC CAG GCC CAG      2016
Ala Cys Val Glu Thr Ala Arg Gln Glu Gln His Glu Ala Gln Ala Gln
            660                 665                 670
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GTT GCA GAG CTA GAG TTG CAG CTG CGG TCT GAG CAG CAA AAA GCA ACT      2064
Val Ala Glu Leu Glu Leu Gln Leu Arg Ser Glu Gln Gln Lys Ala Thr
        675                 680                 685

GAG AAA GAA AGG GTG GCC CAG GAG AAG GAC CAG CTC CAG GAG CAG CTC      2112
Glu Lys Glu Arg Val Ala Gln Glu Lys Asp Gln Leu Gln Glu Gln Leu
    690                 695                 700

CAG GCC CTC AAA GAG TCC TTG AAG GTC ACC AAG GGC AGC CTT GAA GAG      2160
Gln Ala Leu Lys Glu Ser Leu Lys Val Thr Lys Gly Ser Leu Glu Glu
705                 710                 715                 720

GAG AAG CGC AGG GCT GCA GAT GCC CTG GAA GAG CAG CAG CGT TGT ATC      2208
Glu Lys Arg Arg Ala Ala Asp Ala Leu Glu Glu Gln Gln Arg Cys Ile
                725                 730                 735

TCT GAG CTG AAG GCA GAG ACC CGA AGC CTG GTG GAG CAG CAT AAG CGG      2256
Ser Glu Leu Lys Ala Glu Thr Arg Ser Leu Val Glu Gln His Lys Arg
            740                 745                 750

GAA CGA AAG GAG CTG GAA GAA GAG AGG GCT GGG CGC AAG GGG CTG GAG      2304
Glu Arg Lys Glu Leu Glu Glu Glu Arg Ala Gly Arg Lys Gly Leu Glu
        755                 760                 765

GCT CGA TTA CTG CAG CTT GGG GAG GCC CAT CAG GCT GAG ACT GAA GTC      2352
Ala Arg Leu Leu Gln Leu Gly Glu Ala His Gln Ala Glu Thr Glu Val
    770                 775                 780

CTG CGG CGG GAG CTG GCA GAG GCC ATG GCT GCC CAG CAC ACA GCT GAG      2400
Leu Arg Arg Glu Leu Ala Glu Ala Met Ala Ala Gln His Thr Ala Glu
785                 790                 795                 800

AGT GAG TGT GAG CAG CTC GTC AAA GAA GTA GCT GCC TGG CGT GAC GGG      2448
Ser Glu Cys Glu Gln Leu Val Lys Glu Val Ala Ala Trp Arg Asp Gly
                805                 810                 815

TAT GAG GAT AGC CAG CAA GAG GAG GCA CAG TAT GGC GCC ATG TTC CAG      2496
Tyr Glu Asp Ser Gln Gln Glu Glu Ala Gln Tyr Gly Ala Met Phe Gln
            820                 825                 830

GAA CAG CTG ATG ACT TTG AAG GAG GAA TGT GAG AAG GCC CGC CAG GAG      2544
Glu Gln Leu Met Thr Leu Lys Glu Glu Cys Glu Lys Ala Arg Gln Glu
        835                 840                 845

CTG CAG GAG GCA AAG GAG AAG GTG GCA GGC ATA GAA TCC CAC AGC GAG      2592
Leu Gln Glu Ala Lys Glu Lys Val Ala Gly Ile Glu Ser His Ser Glu
    850                 855                 860

CTC CAG ATA AGC CGG CAG CAG AAC AAA CTA GCT GAG CTC CAT GCC AAC      2640
Leu Gln Ile Ser Arg Gln Gln Asn Lys Leu Ala Glu Leu His Ala Asn
865                 870                 875                 880
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CTG GCC AGA GCA CTC CAG CAG GTC CAA GAG AAG GAA GTC AGG GCC CAG      2688
Leu Ala Arg Ala Leu Gln Gln Val Gln Glu Lys Glu Val Arg Ala Gln
                885                 890                 895

AAG CTT GCA GAT GAC CTC TCC ACT CTG CAG GAA AAG ATG GCT GCC ACC      2736
Lys Leu Ala Asp Asp Leu Ser Thr Leu Gln Glu Lys Met Ala Ala Thr
            900                 905                 910

AGC AAA GAG GTG GCC CGC TTG GAG ACC TTG GTC CGC AAG GCA GGT GAG      2784
Ser Lys Glu Val Ala Arg Leu Glu Thr Leu Val Arg Lys Ala Gly Glu
        915                 920                 925

CAG CAG GAA ACA GCC TCC CGG GAG TTA GTC AAG GAG CCT GCG AGG GCA      2832
Gln Gln Glu Thr Ala Ser Arg Glu Leu Val Lys Glu Pro Ala Arg Ala
    930                 935                 940

GGA GAC AGA CAG CCC GAG TGG CTG GAA GAG CAA CAG GGA CGC CAG TTC      2880
Gly Asp Arg Gln Pro Glu Trp Leu Glu Glu Gln Gln Gly Arg Gln Phe
945                 950                 955                 960

TGC AGC ACA CAG GCA GCG CTG CAG GCT ATG GAG CGG GAG GCA GAG CAG      2928
Cys Ser Thr Gln Ala Ala Leu Gln Ala Met Glu Arg Glu Ala Glu Gln
                965                 970                 975

ATG GGC AAT GAG CTG GAA CGG CTG CGG GCC GCG CTG ATG GAG AGC CAG      2976
Met Gly Asn Glu Leu Glu Arg Leu Arg Ala Ala Leu Met Glu Ser Gln
            980                 985                 990

GGG CAG CAG CAG GAG GAG CGT GGG CAG CAG GAA AGG GAG GTG GCG CGG      3024
Gly Gln Gln Gln Glu Glu Arg Gly Gln Gln Glu Arg Glu Val Ala Arg
        995                 1000                1005

CTG ACC CAG GAG CGG GGC CGT GCC CAG GCT GAC CTT GCC CTG GAG AAG      3072
Leu Thr Gln Glu Arg Gly Arg Ala Gln Ala Asp Leu Ala Leu Glu Lys
    1010                1015                1020

GCG GCC AGA GCA GAG CTT GAG ATG CGG CTG CAG AAC GCC CTC AAC GAG      3120
Ala Ala Arg Ala Glu Leu Glu Met Arg Leu Gln Asn Ala Leu Asn Glu
1025                1030                1035                1040

CAG CGT GTG GAG TTC GCT ACC CTG CAA GAG GCA CTG GCT CAT GCC CTG      3168
Gln Arg Val Glu Phe Ala Thr Leu Gln Glu Ala Leu Ala His Ala Leu
                1045                1050                1055

ACG GAA AAG GAA GGC AAG GAC CAG GAG TTG GCC AAG CTT CGT GGT CTG      3216
Thr Glu Lys Glu Gly Lys Asp Gln Glu Leu Ala Lys Leu Arg Gly Leu
            1060                1065                1070

GAG GCA GCC CAG ATA AAA GAG CTG GAG GAA CTT CGG CAA ACC GTG AAG      3264
Glu Ala Ala Gln Ile Lys Glu Leu Glu Glu Leu Arg Gln Thr Val Lys
        1075                1080                1085
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CAA CTG AAG GAA CAG CTG GCT AAG AAA GAA AAG GAG CAC GCA TCT GGC      3312
Gln Leu Lys Glu Gln Leu Ala Lys Lys Glu Lys Glu His Ala Ser Gly
    1090                1095                1100

TCA GGA GCC CAA TCT GAG GCT GCT GGC AGG ACA GAG CCA ACA GGC CCC      3360
Ser Gly Ala Gln Ser Glu Ala Ala Gly Arg Thr Glu Pro Thr Gly Pro
1105                1110                1115                1120

AAG CTG GAA GCA CTG CGG GCA GAG GTG AGC AAG CTG GAA CAG CAA TGC      3408
Lys Leu Glu Ala Leu Arg Ala Glu Val Ser Lys Leu Glu Gln Gln Cys
                1125                1130                1135

CAG AAG CAG CAG GAG CAG GCT GAC AGC CTG GAA CGC AGC CTC GAG GCT      3456
Gln Lys Gln Gln Glu Gln Ala Asp Ser Leu Glu Arg Ser Leu Glu Ala
            1140                1145                1150

GAG CGG GCC TCC CGG GCT GAG CGG GAC AGT GCT CTG GAG ACT CTG CAG      3504
Glu Arg Ala Ser Arg Ala Glu Arg Asp Ser Ala Leu Glu Thr Leu Gln
        1155                1160                1165

GGC CAG TTA GAG GAG AAG GCC CAG GAG CTA GGG CAC AGT CAG AGT GCC      3552
Gly Gln Leu Glu Glu Lys Ala Gln Glu Leu Gly His Ser Gln Ser Ala
    1170                1175                1180

TTA GCC TCG GCC CAA CGG GAG TTG GCT GCC TTC CGC ACC AAG GTA CAA      3600
Leu Ala Ser Ala Gln Arg Glu Leu Ala Ala Phe Arg Thr Lys Val Gln
1185                1190                1195                1200

GAC CAC AGC AAG GCT GAA GAT GAG TGG AAG GCC CAG GTG GCC CGG GGC      3648
Asp His Ser Lys Ala Glu Asp Glu Trp Lys Ala Gln Val Ala Arg Gly
                1205                1210                1215

CGG CAA GAG GCT GAG AGG AAA AAT AGC CTC ATC AGC AGC TTG GAG GAG      3696
Arg Gln Glu Ala Glu Arg Lys Asn Ser Leu Ile Ser Ser Leu Glu Glu
            1220                1225                1230

GAG GTG TCC ATC CTG AAT CGC CAG GTC CTG GAG AAG GAG GGG GAG AGC      3744
Glu Val Ser Ile Leu Asn Arg Gln Val Leu Glu Lys Glu Gly Glu Ser
        1235                1240                1245

AAG GAG TTG AAG CGG CTG GTG ATG GCC GAG TCA GAG AAG AGC CAG AAG      3792
Lys Glu Leu Lys Arg Leu Val Met Ala Glu Ser Glu Lys Ser Gln Lys
    1250                1255                1260

CTG GAG GAG AGC TGC GCC TGC TGC AGG CAG AGA CAG CCA GCA ACA GTG      3840
Leu Glu Glu Ser Cys Ala Cys Cys Arg Gln Arg Gln Pro Ala Thr Val
1265                1270                1275                1280

CCA GAG CTG CAG AAC GCA GCT CTG CTC TGC GGG AGG AGG TGC AGA GCC      3888
Pro Glu Leu Gln Asn Ala Ala Leu Leu Cys Gly Arg Arg Cys Arg Ala
                1285                1290                1295
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TCC GGG AGG GAG GCT GAG AAA CAG CGG GTG GCT TCA GAG AAC CTG CGG      3936
Ser Gly Arg Glu Ala Glu Lys Gln Arg Val Ala Ser Glu Asn Leu Arg
            1300                1305                1310

CAG GAG CTG ACC TCA CAG GCT GAG CGT GCG GAG GAG CTG GGC CAA GAA      3984
Gln Glu Leu Thr Ser Gln Ala Glu Arg Ala Glu Glu Leu Gly Gln Glu
        1315                1320                1325

TTG AAG GCG TGG CAG GAG AAG TTC TTC CAG AAA GAG CAG GCC CTC TCC      4032
Leu Lys Ala Trp Gln Glu Lys Phe Phe Gln Lys Glu Gln Ala Leu Ser
    1330                1335                1340

ACC CTG CAG CTC GAG CAC ACC AGC ACA CAG GCC CTG GTG AGT GAG CTG      4080
Thr Leu Gln Leu Glu His Thr Ser Thr Gln Ala Leu Val Ser Glu Leu
1345                1350                1355                1360

CTG CCA GCT AAG CAC CTC TGC CAG CAG CTG CAG GCC GAG CAG GCC GCT      4128
Leu Pro Ala Lys His Leu Cys Gln Gln Leu Gln Ala Glu Gln Ala Ala
                1365                1370                1375

GCC GAG AAA CGC CAC CGT GAG GAG CTG GAG CAG AGC AAG CAG GCC GCT      4176
Ala Glu Lys Arg His Arg Glu Glu Leu Glu Gln Ser Lys Gln Ala Ala
            1380                1385                1390

GGG GGA CTG CGG GCA GAG CTG CTG CGG GCC CAG CGG GAG CTT GGG GAG      4224
Gly Gly Leu Arg Ala Glu Leu Leu Arg Ala Gln Arg Glu Leu Gly Glu
        1395                1400                1405

CTG ATT CCT CTG CGG CAG AAG GTG GCA GAG CAG GAG CGA ACA GCT CAG      4272
Leu Ile Pro Leu Arg Gln Lys Val Ala Glu Gln Glu Arg Thr Ala Gln
    1410                1415                1420

CAG CTG CGG GCA GAG AAG GCC AGC TAT GCA GAG CAG CTG AGC ATG CTG      4320
Gln Leu Arg Ala Glu Lys Ala Ser Tyr Ala Glu Gln Leu Ser Met Leu
1425                1430                1435                1440

AAG AAG GCG CAT GGC CTG CTG GCA GAG GAG AAC CGG GGG CTG GGT GAG      4368
Lys Lys Ala His Gly Leu Leu Ala Glu Glu Asn Arg Gly Leu Gly Glu
                1445                1450                1455

CGG GCC AAC CTT GGC CGG CAG TTT CTG GAA GTG GAG TTG GAC CAG GCC      4416
Arg Ala Asn Leu Gly Arg Gln Phe Leu Glu Val Glu Leu Asp Gln Ala
            1460                1465                1470

CGG GAA AAG TAT GTC CAA GAG TTG GCA GCC GTA CGT GCT GAT GCT GAG      4464
Arg Glu Lys Tyr Val Gln Glu Leu Ala Ala Val Arg Ala Asp Ala Glu
        1475                1480                1485

ACC CGT CTG GCT GAG GTG CAG CGA GAA GCA CAG AGC ACT GCC CGG GAG      4512
Thr Arg Leu Ala Glu Val Gln Arg Glu Ala Gln Ser Thr Ala Arg Glu
    1490                1495                1500
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CTG GAG GTG ATG ACT GCC AAG TAT GAG GGT GCC AAG GTC AAG GTC CTG      4560
Leu Glu Val Met Thr Ala Lys Tyr Glu Gly Ala Lys Val Lys Val Leu
1505                1510                1515                1520

GAG GAG AGG CAG CGG TTC CAG GAA GAG AGG CAG AAA CTC ACT GCC CAG      4608
Glu Glu Arg Gln Arg Phe Gln Glu Glu Arg Gln Lys Leu Thr Ala Gln
                1525                1530                1535

GTG GAA GAA CTG AGT AAG AAA CTG GCT GAC TCT GAC CAA GCC AGC AAG      4656
Val Glu Glu Leu Ser Lys Lys Leu Ala Asp Ser Asp Gln Ala Ser Lys
            1540                1545                1550

GTG CAG CAG CAG AAG CTG AAG GCT GTC CAG GCT CAG GGA GGC GAG AGC      4704
Val Gln Gln Gln Lys Leu Lys Ala Val Gln Ala Gln Gly Gly Glu Ser
        1555                1560                1565

CAG CAG GAG GCC CAG CGC TTC CAG GCC CAG CTG AAT GAA CTG CAA GCC      4752
Gln Gln Glu Ala Gln Arg Phe Gln Ala Gln Leu Asn Glu Leu Gln Ala
    1570                1575                1580

CAG TTG AGC CAG AAG GAG CAG GCA GCT GAG CAC TAT AAG CTG CAG ATG      4800
Gln Leu Ser Gln Lys Glu Gln Ala Ala Glu His Tyr Lys Leu Gln Met
1585                1590                1595                1600

GAG AAA GCC AAA ACA CAT TAT GAT GCC AAG AAG CAG CAG AAC CAA GAG      4848
Glu Lys Ala Lys Thr His Tyr Asp Ala Lys Lys Gln Gln Asn Gln Glu
                1605                1610                1615

CTG CAG GAG CAG CTG CGG AGC CTG GAG CAG CTG CAG AAG GAA AAC AAA      4896
Leu Gln Glu Gln Leu Arg Ser Leu Glu Gln Leu Gln Lys Glu Asn Lys
            1620                1625                1630

GAG CTG CGA GCT GAA GCT GAA CGG CTG GGC CAT GAG CTA CAG CAG GCT      4944
Glu Leu Arg Ala Glu Ala Glu Arg Leu Gly His Glu Leu Gln Gln Ala
        1635                1640                1645

GGG CTG AAG ACC AAG GAG GCT GAA CAG ACC TGC CGC CAC CTT ACT GCC      4992
Gly Leu Lys Thr Lys Glu Ala Glu Gln Thr Cys Arg His Leu Thr Ala
    1650                1655                1660

CAG GTG CGC AGC CTG GAG GCA CAG GTT GCC CAT GCA GAC CAG CAG CTT      5040
Gln Val Arg Ser Leu Glu Ala Gln Val Ala His Ala Asp Gln Gln Leu
1665                1670                1675                1680

CGA GAC CTG GGC AAA TTC CAG GTG GCA ACT GAT GCT TTA AAG AGC CGT      5088
Arg Asp Leu Gly Lys Phe Gln Val Ala Thr Asp Ala Leu Lys Ser Arg
                1685                1690                1695

GAG CCC CAG GCT AAG CCC CAG CTG GAC TTG AGT ATT GAC AGC CTG GAT      5136
Glu Pro Gln Ala Lys Pro Gln Leu Asp Leu Ser Ile Asp Ser Leu Asp
            1700                1705                1710
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CTG AGC TGC GAG GAG GGG ACC CCA CTC AGT ATC ACC AGC AAG CTG CCT      5184
Leu Ser Cys Glu Glu Gly Thr Pro Leu Ser Ile Thr Ser Lys Leu Pro
        1715                1720                1725

CGT ACC CAG CCA GAC GGC ACC AGC GTC CCT GGA GAA CCA GCC TCA CCT      5232
Arg Thr Gln Pro Asp Gly Thr Ser Val Pro Gly Glu Pro Ala Ser Pro
    1730                1735                1740

ATC TCC CAG CGC CTG CCC CCC AAG GTA GAA TCC CTG GAG AGT CTC TAC      5280
Ile Ser Gln Arg Leu Pro Pro Lys Val Glu Ser Leu Glu Ser Leu Tyr
1745                1750                1755                1760

TTC ACT CCC ATC CCT GCT CGG AGT CAG GCC CCC CTG GAG AGC AGC CTG      5328
Phe Thr Pro Ile Pro Ala Arg Ser Gln Ala Pro Leu Glu Ser Ser Leu
                1765                1770                1775

GAC TCC CTG GGA GAC GTC TTC CTG GAC TCG GGT CGT AAG ACC CGC TCC      5376
Asp Ser Leu Gly Asp Val Phe Leu Asp Ser Gly Arg Lys Thr Arg Ser
            1780                1785                1790

GCT CGT CGG CGC ACC ACG CAG ATC ATC AAC ATC ACC ATG ACC AAG AAG      5424
Ala Arg Arg Arg Thr Thr Gln Ile Ile Asn Ile Thr Met Thr Lys Lys
        1795                1800                1805

CTA GAT GTG GAA GAG CCA GAC AGC GCC AAC TCA TCG TTC TAC AGC ACG      5472
Leu Asp Val Glu Glu Pro Asp Ser Ala Asn Ser Ser Phe Tyr Ser Thr
    1810                1815                1820

CGG TCT GCT CCT GCT TCC CAG GCT AGC CTG CGA GCC ACC TCC TCT ACT      5520
Arg Ser Ala Pro Ala Ser Gln Ala Ser Leu Arg Ala Thr Ser Ser Thr
1825                1830                1835                1840

CAG TCT CTA GCT CGC CTG GGT TCT CCC GAT TAT GGC AAC TCA GCC CTG      5568
Gln Ser Leu Ala Arg Leu Gly Ser Pro Asp Tyr Gly Asn Ser Ala Leu
                1845                1850                1855

CTC AGC TTG CCT GGC TAC CGC CCC ACC ACT CGC AGT TCT GCT CGT CGT      5616
Leu Ser Leu Pro Gly Tyr Arg Pro Thr Thr Arg Ser Ser Ala Arg Arg
            1860                1865                1870

TCC CAG GCC GGG GTG TCC AGT GGG GCC CCT CCA GGA AGG AAC AGC TTC      5664
Ser Gln Ala Gly Val Ser Ser Gly Ala Pro Pro Gly Arg Asn Ser Phe
        1875                1880                1885

TAC ATG GGC ACT TGC CAG GAT GAG CCT GAG CAG CTG GAT GAC TGG AAC      5712
Tyr Met Gly Thr Cys Gln Asp Glu Pro Glu Gln Leu Asp Asp Trp Asn
    1890                1895                1900

CGC ATT GCA GAG CTG CAG CAG CGC AAT CGA GTG TGC CCC CCA CAT CTG      5760
Arg Ile Ala Glu Leu Gln Gln Arg Asn Arg Val Cys Pro Pro His Leu
1905                1910                1915                1920
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AAG ACC TGC TAT CCC CTG GAG TCC AGG CCT TCC CTG AGC CTG GGC ACC      5808
Lys Thr Cys Tyr Pro Leu Glu Ser Arg Pro Ser Leu Ser Leu Gly Thr
                1925                1930                1935

ATC ACA GAT GAG GAG ATG AAA ACT GGA GAC CCC CAA GAG ACC CTG CGC      5856
Ile Thr Asp Glu Glu Met Lys Thr Gly Asp Pro Gln Glu Thr Leu Arg
            1940                1945                1950

CGA GCC AGC ATG CAG CCA ATC CAG ATA GCC GAG GGC ACT GGC ATC ACC      5904
Arg Ala Ser Met Gln Pro Ile Gln Ile Ala Glu Gly Thr Gly Ile Thr
        1955                1960                1965

ACC CGG CAG CAG CGC AAA CGG GTC TCC CTA GAG CCC CAC CAG GGC CCT      5952
Thr Arg Gln Gln Arg Lys Arg Val Ser Leu Glu Pro His Gln Gly Pro
    1970                1975                1980

GGA ACT CCT GAG TCT AAG AAG GCC ACC AGC TGT TTC CCA CGC CCC ATG      6000
Gly Thr Pro Glu Ser Lys Lys Ala Thr Ser Cys Phe Pro Arg Pro Met
1985                1990                1995                2000

ACT CCC CGA GAC CGA CAT GAA GGG CGC AAA CAG AGC ACT ACT GAG GCC      6048
Thr Pro Arg Asp Arg His Glu Gly Arg Lys Gln Ser Thr Thr Glu Ala
                2005                2010                2015

CAG AAG AAA GCA GCT CCA GCT TCT ACT AAA CAG GCT GAC CGG CGC CAG      6096
Gln Lys Lys Ala Ala Pro Ala Ser Thr Lys Gln Ala Asp Arg Arg Gln
            2020                2025                2030

TCG ATG GCC TTC AGC ATC CTC AAC ACA CCC AAG AAG CTA GGG AAC AGC      6144
Ser Met Ala Phe Ser Ile Leu Asn Thr Pro Lys Lys Leu Gly Asn Ser
        2035                2040                2045

CTT CTG CGG CGG GGA GCC TCA AAG AAG GCC CTG TCC AAG GCT TCC CCC      6192
Leu Leu Arg Arg Gly Ala Ser Lys Lys Ala Leu Ser Lys Ala Ser Pro
    2050                2055                2060

AAC ACT CGC AGT GGA ACC CGC CGT TCT CCG CGC ATT GCC ACC ACC ACA      6240
Asn Thr Arg Ser Gly Thr Arg Arg Ser Pro Arg Ile Ala Thr Thr Thr
2065                2070                2075                2080

GCC AGT GCC GCC ACT GCT GCC GCC ATT GGT GCC ACC CCT CGA GCC AAG      6288
Ala Ser Ala Ala Thr Ala Ala Ala Ile Gly Ala Thr Pro Arg Ala Lys
                2085                2090                2095

GGC AAG GCA AAG CAC TAA                                             6306
Gly Lys Ala Lys His
            2100
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2101 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Thr Leu His Ala Thr Arg Gly Ala Ala Leu Leu Ser Trp Val Asn
 1               5                  10                  15

Ser Leu His Val Ala Asp Pro Val Glu Ala Val Leu Gln Leu Gln Asp
            20                  25                  30

Cys Ser Ile Phe Ile Lys Ile Ile Asp Arg Ile His Gly Thr Glu Glu
        35                  40                  45

Gly Gln Gln Ile Leu Lys Gln Pro Val Ser Glu Arg Leu Asp Phe Val
    50                  55                  60

Cys Ser Phe Leu Gln Lys Asn Arg Lys His Pro Ser Ser Pro Glu Cys
65                  70                  75                  80

Leu Val Ser Ala Gln Lys Val Leu Glu Gly Ser Glu Leu Glu Leu Ala
                85                  90                  95

Lys Met Thr Met Leu Leu Leu Tyr His Ser Thr Met Ser Ser Lys Ser
            100                 105                 110

Pro Arg Asp Trp Glu Gln Phe Glu Tyr Lys Ile Gln Ala Glu Leu Ala
        115                 120                 125

Val Ile Leu Lys Phe Val Leu Asp His Glu Asp Gly Leu Asn Leu Asn
    130                 135                 140

Glu Asp Leu Glu Asn Phe Leu Gln Lys Ala Pro Val Pro Ser Thr Cys
145                 150                 155                 160

Ser Ser Thr Phe Pro Glu Glu Leu Ser Pro Pro Ser His Gln Ala Lys
                165                 170                 175

Arg Glu Ile Arg Phe Leu Glu Leu Gln Lys Val Ala Ser Ser Ser Ser
            180                 185                 190

Gly Asn Asn Phe Leu Ser Gly Ser Pro Ala Ser Pro Met Gly Asp Ile
        195                 200                 205

Leu Gln Thr Pro Gln Phe Gln Met Arg Arg Leu Lys Lys Gln Leu Ala
    210                 215                 220
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Asp Glu Arg Ser Asn Arg Asp Glu Leu Glu Leu Glu Leu Ala Glu Asn
225                 230                 235                 240

Arg Lys Leu Leu Thr Glu Lys Asp Ala Gln Ile Ala Met Met Gln Gln
                245                 250                 255

Arg Ile Asp Arg Leu Ala Leu Leu Asn Glu Lys Gln Ala Ala Ser Pro
            260                 265                 270

Leu Glu Pro Lys Glu Leu Glu Glu Leu Arg Asp Lys Asn Glu Ser Leu
        275                 280                 285

Thr Met Arg Leu His Glu Thr Leu Lys Gln Cys Gln Asp Leu Lys Thr
    290                 295                 300

Glu Lys Ser Gln Met Asp Arg Lys Ile Asn Gln Leu Ser Glu Glu Asn
305                 310                 315                 320

Gly Asp Leu Ser Phe Lys Leu Arg Glu Phe Ala Ser His Leu Gln Gln
                325                 330                 335

Leu Gln Asp Ala Leu Asn Glu Leu Thr Glu Glu His Ser Lys Ala Thr
            340                 345                 350

Gln Glu Trp Leu Glu Lys Gln Ala Gln Leu Glu Lys Glu Leu Ser Ala
        355                 360                 365

Ala Leu Gln Asp Lys Lys Cys Leu Glu Glu Lys Asn Glu Ile Leu Gln
    370                 375                 380

Gly Lys Leu Ser Gln Leu Glu Glu His Leu Ser Gln Leu Gln Asp Asn
385                 390                 395                 400

Pro Pro Gln Glu Lys Gly Glu Val Leu Gly Asp Val Leu Gln Leu Glu
                405                 410                 415

Thr Leu Lys Gln Glu Ala Ala Thr Leu Ala Ala Asn Asn Thr Gln Leu
            420                 425                 430

Gln Ala Arg Val Glu Met Leu Glu Thr Glu Arg Gly Gln Gln Glu Ala
        435                 440                 445

Lys Leu Leu Ala Glu Arg Gly His Phe Glu Glu Glu Lys Gln Gln Leu
    450                 455                 460

Ser Ser Leu Ile Thr Asp Leu Gln Ser Ser Ile Ser Asn Leu Ser Gln
465                 470                 475                 480

Ala Lys Glu Glu Leu Glu Gln Ala Ser Gln Ala His Gly Ala Arg Leu
                485                 490                 495
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Thr Ala Gln Val Ala Ser Leu Thr Ser Glu Leu Thr Thr Leu Asn Ala
            500                 505                 510

Thr Ile Gln Gln Gln Asp Gln Glu Leu Ala Gly Leu Lys Gln Gln Ala
        515                 520                 525

Lys Glu Lys Gln Ala Gln Leu Ala Gln Thr Leu Gln Gln Gln Glu Gln
    530                 535                 540

Ala Ser Gln Gly Leu Arg His Gln Val Glu Gln Leu Ser Ser Ser Leu
545                 550                 555                 560

Lys Gln Lys Glu Gln Gln Leu Lys Glu Val Ala Glu Lys Gln Glu Ala
                565                 570                 575

Thr Arg Gln Asp His Ala Gln Gln Leu Ala Thr Ala Ala Glu Glu Arg
            580                 585                 590

Glu Ala Ser Leu Arg Glu Arg Asp Ala Ala Leu Lys Gln Leu Glu Ala
        595                 600                 605

Leu Glu Lys Glu Lys Ala Ala Lys Leu Glu Ile Leu Gln Gln Gln Leu
    610                 615                 620

Gln Val Ala Asn Glu Ala Arg Asp Ser Ala Gln Thr Ser Val Thr Gln
625                 630                 635                 640

Ala Gln Arg Glu Lys Ala Glu Leu Ser Arg Lys Val Glu Glu Leu Gln
                645                 650                 655

Ala Cys Val Glu Thr Ala Arg Gln Glu Gln His Glu Ala Gln Ala Gln
            660                 665                 670

Val Ala Glu Leu Glu Leu Gln Leu Arg Ser Glu Gln Gln Lys Ala Thr
        675                 680                 685

Glu Lys Glu Arg Val Ala Gln Glu Lys Asp Gln Leu Gln Glu Gln Leu
    690                 695                 700

Gln Ala Leu Lys Glu Ser Leu Lys Val Thr Lys Gly Ser Leu Glu Glu
705                 710                 715                 720

Glu Lys Arg Arg Ala Ala Asp Ala Leu Glu Glu Gln Gln Arg Cys Ile
                725                 730                 735

Ser Glu Leu Lys Ala Glu Thr Arg Ser Leu Val Glu Gln His Lys Arg
            740                 745                 750

Glu Arg Lys Glu Leu Glu Glu Glu Arg Ala Gly Arg Lys Gly Leu Glu
        755                 760                 765
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Ala Arg Leu Leu Gln Leu Gly Glu Ala His Gln Ala Glu Thr Glu Val
    770                 775                 780

Leu Arg Arg Glu Leu Ala Glu Ala Met Ala Ala Gln His Thr Ala Glu
785                 790                 795                 800

Ser Glu Cys Glu Gln Leu Val Lys Glu Val Ala Ala Trp Arg Asp Gly
                805                 810                 815

Tyr Glu Asp Ser Gln Gln Glu Glu Ala Gln Tyr Gly Ala Met Phe Gln
            820                 825                 830

Glu Gln Leu Met Thr Leu Lys Glu Glu Cys Glu Lys Ala Arg Gln Glu
        835                 840                 845

Leu Gln Glu Ala Lys Glu Lys Val Ala Gly Ile Glu Ser His Ser Glu
    850                 855                 860

Leu Gln Ile Ser Arg Gln Gln Asn Lys Leu Ala Glu Leu His Ala Asn
865                 870                 875                 880

Leu Ala Arg Ala Leu Gln Gln Val Gln Glu Lys Glu Val Arg Ala Gln
                885                 890                 895

Lys Leu Ala Asp Asp Leu Ser Thr Leu Gln Glu Lys Met Ala Ala Thr
            900                 905                 910

Ser Lys Glu Val Ala Arg Leu Glu Thr Leu Val Arg Lys Ala Gly Glu
        915                 920                 925

Gln Gln Glu Thr Ala Ser Arg Glu Leu Val Lys Glu Pro Ala Arg Ala
    930                 935                 940

Gly Asp Arg Gln Pro Glu Trp Leu Glu Glu Gln Gln Gly Arg Gln Phe
945                 950                 955                 960

Cys Ser Thr Gln Ala Ala Leu Gln Ala Met Glu Arg Glu Ala Glu Gln
                965                 970                 975

Met Gly Asn Glu Leu Glu Arg Leu Arg Ala Ala Leu Met Glu Ser Gln
            980                 985                 990

Gly Gln Gln Gln Glu Glu Arg Gly Gln Gln Glu Arg Glu Val Ala Arg
        995                 1000                1005

Leu Thr Gln Glu Arg Gly Arg Ala Gln Ala Asp Leu Ala Leu Glu Lys
    1010                1015                1020

Ala Ala Arg Ala Glu Leu Glu Met Arg Leu Gln Asn Ala Leu Asn Glu
1025                1030                1035                1040
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Gln Arg Val Glu Phe Ala Thr Leu Gln Glu Ala Leu Ala His Ala Leu
1045 1050 1055

Thr Glu Lys Glu Gly Lys Asp Gln Glu Leu Ala Lys Leu Arg Gly Leu
1060 1065 1070

Glu Ala Ala Gln Ile Lys Glu Leu Glu Glu Leu Arg Gln Thr Val Lys
1075 1080 1085

Gln Leu Lys Glu Gln Leu Ala Lys Lys Glu Lys Glu His Ala Ser Gly
1090 1095 1100

Ser Gly Ala Gln Ser Glu Ala Ala Gly Arg Thr Glu Pro Thr Gly Pro
1105 1110 1115 1120

Lys Leu Glu Ala Leu Arg Ala Glu Val Ser Lys Leu Glu Gln Gln Cys
1125 1130 1135

Gln Lys Gln Gln Glu Gln Ala Asp Ser Leu Glu Arg Ser Leu Glu Ala
1140 1145 1150

Glu Arg Ala Ser Arg Ala Glu Arg Asp Ser Ala Leu Glu Thr Leu Gln
1155 1160 1165

Gly Gln Leu Glu Glu Lys Ala Gln Glu Leu Gly His Ser Gln Ser Ala
1170 1175 1180

Leu Ala Ser Ala Gln Arg Glu Leu Ala Ala Phe Arg Thr Lys Val Gln
1185 1190 1195 1200

Asp His Ser Lys Ala Glu Asp Glu Trp Lys Ala Gln Val Ala Arg Gly
1205 1210 1215

Arg Gln Glu Ala Glu Arg Lys Asn Ser Leu Ile Ser Ser Leu Glu Glu
1220 1225 1230

Glu Val Ser Ile Leu Asn Arg Gln Val Leu Glu Lys Glu Gly Glu Ser
1235 1240 1245

Lys Glu Leu Lys Arg Leu Val Met Ala Glu Ser Glu Lys Ser Gln Lys
1250 1255 1260

Leu Glu Glu Ser Cys Ala Cys Cys Arg Gln Arg Gln Pro Ala Thr Val
1265 1270 1275 1280

Pro Glu Leu Gln Asn Ala Ala Leu Leu Cys Gly Arg Arg Cys Arg Ala
1285 1290 1295

Ser Gly Arg Glu Ala Glu Lys Gln Arg Val Ala Ser Glu Asn Leu Arg
1300 1305 1310

Gln Glu Leu Thr Ser Gln Ala Glu Arg Ala Glu Glu Leu Gly Gln Glu
1315 1320 1325

Leu Lys Ala Trp Gln Glu Lys Phe Phe Gln Lys Glu Gln Ala Leu Ser
1330 1335 1340

Thr Leu Gln Leu Glu His Thr Ser Thr Gln Ala Leu Val Ser Glu Leu
1345 1350 1355 1360

Leu Pro Ala Lys His Leu Cys Gln Gln Leu Gln Ala Glu Gln Ala Ala
1365 1370 1375

Ala Glu Lys Arg His Arg Glu Glu Leu Glu Gln Ser Lys Gln Ala Ala
1380 1385 1390

Gly Gly Leu Arg Ala Glu Leu Leu Arg Ala Gln Arg Glu Leu Gly Glu
1395 1400 1405

Leu Ile Pro Leu Arg Gln Lys Val Ala Glu Gln Glu Arg Thr Ala Gln
1410 1415 1420

Gln Leu Arg Ala Glu Lys Ala Ser Tyr Ala Glu Gln Leu Ser Met Leu
1425 1430 1435 1440

Lys Lys Ala His Gly Leu Leu Ala Glu Glu Asn Arg Gly Leu Gly Glu
1445 1450 1455

Arg Ala Asn Leu Gly Arg Gln Phe Leu Glu Val Glu Leu Asp Gln Ala
1460 1465 1470

Arg Glu Lys Tyr Val Gln Glu Leu Ala Ala Val Arg Ala Asp Ala Glu
1475 1480 1485

Thr Arg Leu Ala Glu Val Gln Arg Glu Ala Gln Ser Thr Ala Arg Glu
1490 1495 1500

Leu Glu Val Met Thr Ala Lys Tyr Glu Gly Ala Lys Val Lys Val Leu
1505 1510 1515 1520

Glu Glu Arg Gln Arg Phe Gln Glu Glu Arg Gln Lys Leu Thr Ala Gln
1525 1530 1535

Val Glu Glu Leu Ser Lys Lys Leu Ala Asp Ser Asp Gln Ala Ser Lys
1540 1545 1550

Val Gln Gln Gln Lys Leu Lys Ala Val Gln Ala Gln Gly Gly Glu Ser
1555 1560 1565

Gln Gln Glu Ala Gln Arg Phe Gln Ala Gln Leu Asn Glu Leu Gln Ala
1570 1575 1580

Gln Leu Ser Gln Lys Glu Gln Ala Ala Glu His Tyr Lys Leu Gln Met
1585 1590 1595 1600

Glu Lys Ala Lys Thr His Tyr Asp Ala Lys Lys Gln Gln Asn Gln Glu
1605 1610 1615

Leu Gln Glu Gln Leu Arg Ser Leu Glu Gln Leu Gln Lys Glu Asn Lys
1620 1625 1630

Glu Leu Arg Ala Glu Ala Glu Arg Leu Gly His Glu Leu Gln Gln Ala
1635 1640 1645

Gly Leu Lys Thr Lys Glu Ala Glu Gln Thr Cys Arg His Leu Thr Ala
1650 1655 1660

Gln Val Arg Ser Leu Glu Ala Gln Val Ala His Ala Asp Gln Gln Leu
1665 1670 1675 1680

Arg Asp Leu Gly Lys Phe Gln Val Ala Thr Asp Ala Leu Lys Ser Arg
1685 1690 1695

Glu Pro Gln Ala Lys Pro Gln Leu Asp Leu Ser Ile Asp Ser Leu Asp
1700 1705 1710

Leu Ser Cys Glu Glu Gly Thr Pro Leu Ser Ile Thr Ser Lys Leu Pro
1715 1720 1725

Arg Thr Gln Pro Asp Gly Thr Ser Val Pro Gly Glu Pro Ala Ser Pro
1730 1735 1740

Ile Ser Gln Arg Leu Pro Pro Lys Val Glu Ser Leu Glu Ser Leu Tyr
1745 1750 1755 1760

Phe Thr Pro Ile Pro Ala Arg Ser Gln Ala Pro Leu Glu Ser Ser Leu
1765 1770 1775

Asp Ser Leu Gly Asp Val Phe Leu Asp Ser Gly Arg Lys Thr Arg Ser
1780 1785 1790

Ala Arg Arg Arg Thr Thr Gln Ile Ile Asn Ile Thr Met Thr Lys Lys
1795 1800 1805

Leu Asp Val Glu Glu Pro Asp Ser Ala Asn Ser Ser Phe Tyr Ser Thr
1810 1815 1820

Arg Ser Ala Pro Ala Ser Gln Ala Ser Leu Arg Ala Thr Ser Ser Thr
1825 1830 1835 1840

Gln Ser Leu Ala Arg Leu Gly Ser Pro Asp Tyr Gly Asn Ser Ala Leu
1845 1850 1855

Leu Ser Leu Pro Gly Tyr Arg Pro Thr Thr Arg Ser Ser Ala Arg Arg
1860 1865 1870

Ser Gln Ala Gly Val Ser Ser Gly Ala Pro Pro Gly Arg Asn Ser Phe
1875 1880 1885

Tyr Met Gly Thr Cys Gln Asp Glu Pro Glu Gln Leu Asp Asp Trp Asn
1890 1895 1900

Arg Ile Ala Glu Leu Gln Gln Arg Asn Arg Val Cys Pro Pro His Leu
1905 1910 1915 1920

Lys Thr Cys Tyr Pro Leu Glu Ser Arg Pro Ser Leu Ser Leu Gly Thr
1925 1930 1935

Ile Thr Asp Glu Glu Met Lys Thr Gly Asp Pro Gln Glu Thr Leu Arg
1940 1945 1950

Arg Ala Ser Met Gln Pro Ile Gln Ile Ala Glu Gly Thr Gly Ile Thr
1955 1960 1965

Thr Arg Gln Gln Arg Lys Arg Val Ser Leu Glu Pro His Gln Gly Pro
1970 1975 1980

Gly Thr Pro Glu Ser Lys Lys Ala Thr Ser Cys Phe Pro Arg Pro Met
1985 1990 1995 2000

Thr Pro Arg Asp Arg His Glu Gly Arg Lys Gln Ser Thr Thr Glu Ala
2005 2010 2015

Gln Lys Lys Ala Ala Pro Ala Ser Thr Lys Gln Ala Asp Arg Arg Gln
2020 2025 2030

Ser Met Ala Phe Ser Ile Leu Asn Thr Pro Lys Lys Leu Gly Asn Ser
2035 2040 2045

Leu Leu Arg Arg Gly Ala Ser Lys Lys Ala Leu Ser Lys Ala Ser Pro
2050 2055 2060

Asn Thr Arg Ser Gly Thr Arg Arg Ser Pro Arg Ile Ala Thr Thr Thr
2065 2070 2075 2080

Ala Ser Ala Ala Thr Ala Ala Ala Ile Gly Ala Thr Pro Arg Ala Lys
2085 2090 2095

Gly Lys Ala Lys His
2100

(2) INFORMATION FOR SEQ ID NO:5:

(i)SEQUENCE CHARACTERISTICS:
(A) LENGTH: 353 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii)MOLECULE TYPE: DNA

(ix)FEATURE:
(A) NAME/KEY: mRNA
(B) LOCATION: 1..353
(D) OTHER INFORMATION: /note= "ANTI-SENSE SEQUENCE TO PART OF THE MT1 MRNA TRANSCRIPT: N TERMINUS OF PROTEN CODING SEQUENCE AND UPSTREAM 53 NUCLEOTIDES."

(ix)FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (298..300)
(D) OTHER INFORMATION: /note= "MT1 INITIATION CODON SEQUENCE ON COMPLEMENTARY STRAND."

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCAATTTTA ACTTGTTCTT GTTTTTCTCG TTGTGCAAGG CGAGCTGCAA CTTCTTCAGG 60

TGGTCGCTCC CTTATAGAAG ATGAGGATGC TTCTGAAAGT GCAGGTGTGG GTTTTCCTTC 120

ACCAATTTCA GGGTGATCAG TTTTTAAAGA TTCCTCAGGC TGAACTGCAG GGGCTGGGAC 180

CGACAGGGTA TCACCTGCTG CAGAAATAAT TTGAGCCGCT TCTGTAGGTG CTGTTGCTGA 240

AGCTGGAGTA TCTCCCTTTT GTTGTTGGAG TTGTGAGGCA GGCTGTTTAG ATTCTTTCAT 300

TACTTCTGAT ACACTAGAGA TTTTTAGTGG ACCCGACTGA ATCGATTTCT TTG 353

(2) INFORMATION FOR SEQ ID NO:6:

(i)SEQUENCE CHARACTERISTICS:
(A) LENGTH: 348 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii)MOLECULE TYPE: DNA

(ix)FEATURE:
(A) NAME/KEY: mRNA
(B) LOCATION: 1..348
(D) OTHER INFORMATION: /note= "ANTISENSE SEQUENCE TO PART OF MT2 TRANSCRIPT: N TERMINUS OF PROTEIN CODING REGION AND UPSTREAM 48 NUCLEOTIDES."

(ix)FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (298..300)
(D) OTHER INFORMATION: /note= "MT2 INITIATION CODON SEQUENCE ON COMPLEMENTARY STRAND."

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:6:

CATGGTCATC TTCGCCAGTT CCAGCTCTGA TCCCTCTAGC ACCTTCTGTG CAGATACCAG 60

GCGTTCTGGG GAAGAGGGAT GTTTTCGATT TTTCTGCAGA AAACTGCACA CAAAGTCCAG 120

TCTCTCTGAC ACCGGCTGCT TCTTGATTTG CTGTCCCTCT TCAGTGCCAT GGATTCTGTC 180

AATGATCTTG ATGAAGATGC TGCAGTCCTG GAGCTGCAGC ACAGCCTCCA CAGGGTCAGC 240

CACGTGTAGA CTGTTCACCC AAGAGAGGAG TGCAGCCCCC CGGGTGGCGT GGAGTGTCAT 300

CTTGGTGATG CCAGACAGTC ACTCCAATGC GCCTGTAATC CCAGCTAC 348

What is claimed is:

1. An isolated nucleic acid comprising the DNA sequence of Seq. ID No. 1, including variants thereof.

2. An isolated nucleic acid that hybridizes with the DNA sequence of Seq. ID No. 1 under stringent hybridization conditions.

3. A host cell transfected with the nucleic acid of claim 1 or 2.

4. A vector comprising the nucleic acid of claim 1 or 2.

5. A protein or protein fragment encoded by the DNA sequence of Seq. ID No. 1, including variants thereof, in combination with an adjuvant.

6. A protein, produced by recombinant DNA in a host cell and isolated from said host cell, said recombinant DNA having the sequence of Seq. ID No. 1, including variants thereof.

7. A binding protein that binds to an epitope on the protein of claim 6.

8. The binding protein of claim 7 wherein said binding protein is an antibody or an antibody fragment.

9. A method of manufacturing an antibody for use in the detection of abnormal cell types, the method comprising the steps of:

a) combining a recombinantly-produced protein or protein fragment encoded by the DNA of Seq. ID No. 1 or 3, including a variant thereof, with an adjuvant to form a composition suitable for injection into a mammal;

b) injecting the composition into a mammal to induce antibody production in said mammal against said recombinantly-produced protein or protein fragment; and

c) isolating said antibody from said mammal.

10. The method of claim 9 wherein said step of isolating said antibody from said mammal is performed by isolating from said mammal a cell producing said antibody.

11. A method of detecting an abnormal cell type in a sample containing cells or cell nucleus debris, the method comprising the steps of:

(a) contacting the sample with a binding protein that recognizes an epitope on a marker protein comprising an amino acid sequence encoded by the DNA of Seq. ID No. 1 or 3 or a variant thereof; and

(b) detecting the presence in the sample of said marker protein or a fragment thereof.

12. The method of claim 9 or 11 wherein said abnormal cell type is a malignant cell type.

13. The method of claim 12 wherein said malignant cell type is characteristic of a malignant bladder, breast, prostate, lung, colon, ovary or cervix cell type.

14. The method of claim 11 wherein said binding protein is an antibody that binds specifically to an epitope on said marker protein or protein fragment.

15. The method of claim 14 wherein said antibody has a binding affinity for said epitope greater than $10^5 M^{-1}$.

16. The method of claim 15 wherein said antibody has a binding affinity greater than $10^7 M^{-1}$.

17. The method of claim 11 comprising the additional step of quantitating the abundance of said marker protein in said sample.

18. The method of claim 11 wherein said sample comprises a body fluid.

19. The method of claim 18 wherein said body fluid is selected from the group consisting of serum, plasma, blood, urine, semen, vaginal secretions, spinal fluid, ascitic fluid, peritoneal fluid, sputum, and breast exudate.

20. A method for determining the degree of cell death in a tissue, the method comprising the steps of:

(a) contacting a sample with a binding protein that recognizes an epitope on a marker protein for cell death, said marker protein comprising an amino acid sequence encoded by the DNA of Seq. ID No. 1 or 3 or a variant thereof; and

(b) detecting the concentration of said marker protein or protein fragment released from the cells of said tissue, said marker protein or protein fragment comprising an amino acid sequence encoded by the DNA sequence of Seq. ID No. 1 or 3 or a variant thereof,

the concentration of said marker protein or protein fragment detected being indicative of the degree of cell death in said tissue.

21. The method of claim 20 comprising the additional steps of:

c) repeating, at intervals, the steps of detecting the concentration of said marker protein or protein fragments thereof; and

d) comparing said detected concentrations, wherein changes in said detected concentrations are indicative of the status of said tissue.

22. The method of claim 20 for use in monitoring change in the status of a disease or the efficacy of a therapy, wherein a decrease in said detected concentrations is indicative of a decrease in cell death, and an increase in said detected concentrations is indicative of an increase in cell death.

23. the method of claim 20 wherein said tissue is characteristic of breast, prostate, lung, colon, ovary, bladder or cervical tissue.

24. A method of detecting an abnormal cell type in a sample containing cells or cell nucleus debris, the method comprising the steps of:

a) contacting the sample with a nucleic acid that hybridizes specifically to an mRNA transcript encoded by the DNA sequence of Seq. ID No. 1 or Seq. 3, said transcript, when translated, encoding the amino acid sequence of Seq. ID No 1 or Seq. ID No. 3 or a variant thereof; and

b) detecting the presence in the sample of said mRNA transcript or a fragment or variant thereof.

25. The method of claim 24 wherein said abnormal cell type is a malignant cell type.

26. The method of claim 25 wherein said malignant cell type is characteristic of a malignant breast, prostate, lung, colon, cervix or bladder cell type.

27. The method of claim 24 wherein said nucleic acid hybridizes with said mRNA transcript under stringent hybridization conditions.

28. The method of claim 24 comprising the additional step of quantitating the abundance of said transcript in said sample.

29. Use of a molecule capable of binding to the mRNA transcript or protein product of MT1 or MT2, including variants thereof, for the manufacture of a cancer therapeutic agent.

30. Use according to claim 29 wherein said canor therapeutic agent is for the treatment of breast, prostate, cervix, ovarian, bladder, colon, prostate or lung cancer.

31. Use according to claim 30 wherein said molecule is an oligonucleotide complementary to at least a portion of the DNA sequence of Seq. ID No. 1 or 3.

32. Use according to claim 31 wherein said oligonucleotide is a synthetic oligonucleotide and comprises at least a portion of the sequence of Seq. ID No. 5 or 6.

33. Use according to claim 29 wherein said molecule is a member of a binding pair capable of binding MT1 or MT2 or a variant thereof substantially irreversibly.

34. Use according to claim 33 wherein said member of said binding pair binds MT1 or MT2 or a variant thereof with an affinity greater than about 10^9 M^{-1}.

35. A synthetic oligonucleotide in admixture with a pharmaceutical carrier for use in the manufacture of a therapeutic agent, said synthetic oligonucleotide comprising a sequence complementary to at least a portion of the mRNA transcript of MT1 or MT2 or a variant thereof.

36. The synthetic oligonucleotide of claim 35 comprising a sequence complementary to at least a portion of the DNA sequence of Seq. ID No. 1 or 3 or a variant thereof.

37. The synthetic oligonucleotide of claim 35 comprising at least a portion of the sequence of Seq. ID No. 5 or 6 or a variant thereof.

38. The synthetic oligonucletide of claim 35 being at least 15 nucleotides in length.

39. A binding protein for use in the manufacture of a medicament, said binding protein having a binding affinity of greater than about $10^9 M^{-1}$ for the protein encoded by the DNA of Seq. ID No. 1 or 3, or a variant thereof.

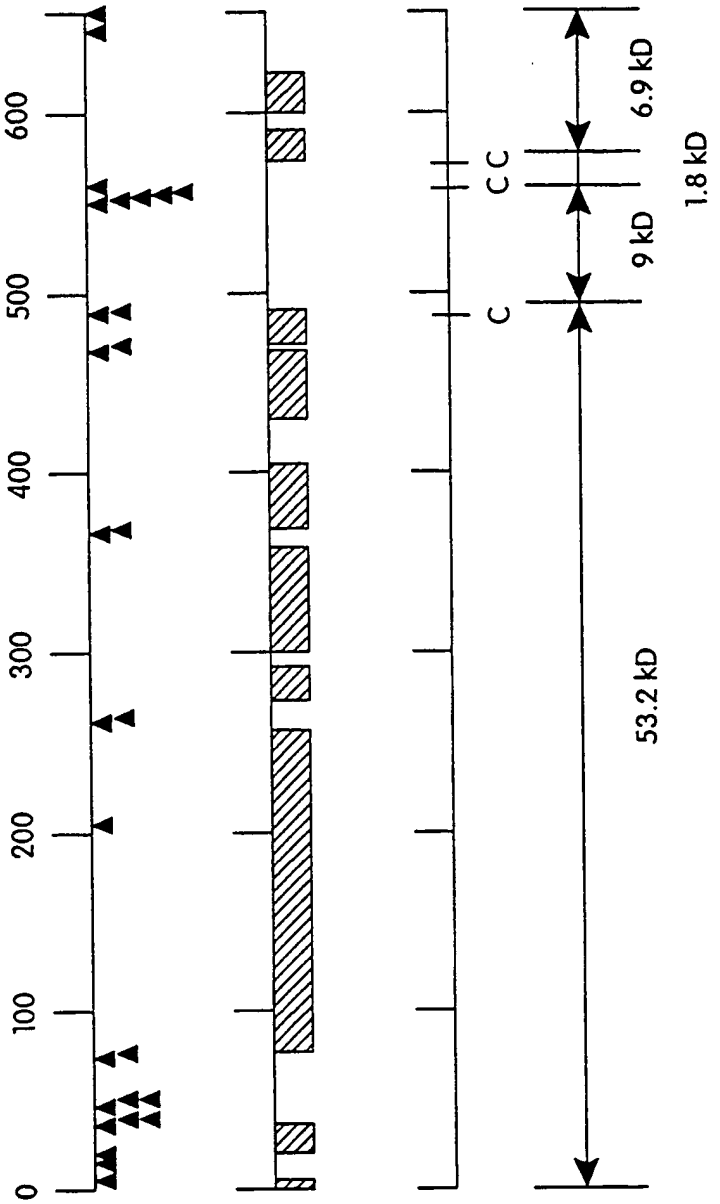

Fig. 1A Fig. 1B Fig. 1C Fig. 1D

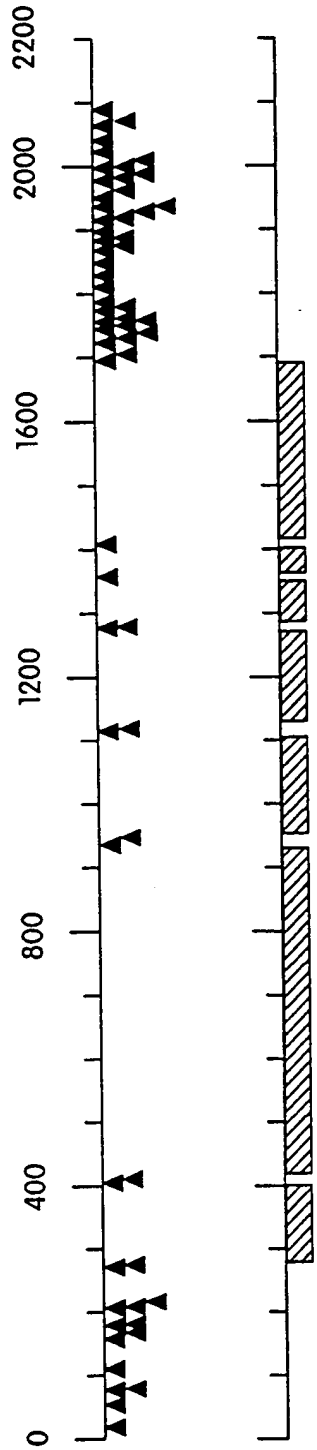

Fig. 2A Fig. 2B

FIG. 3.1

SAMPLE	SAMPLE #	ANTIBODY COMBINATIONS 302-22 302-18	302-33 107-7	302-29 302-18	302-29 107-7
NORMAL	1	0.0	0.0	0.0	0.0
NORMAL	2	0.0	0.0	0.0	0.0
NORMAL	3	0.0	0.0	0.0	0.0
NORMAL	4	0.0	0.0	0.0	0.0
NORMAL	5	0.0	0.0	0.0	0.0
NORMAL	6	0.0	0.0	0.0	0.0
NORMAL	7	0.0	0.0	0.0	0.0
NORMAL	8	0.0	0.0	0.0	0.0
NORMAL	9	0.0	0.0	0.5	0.0
NORMAL	10	0.0	0.7	1.2	0.0
NORMAL	11	0.0	0.0	0.0	0.0
NORMAL	12	0.0	0.0	0.0	0.2
NORMAL	13	0.0	0.7	0.0	0.3
NORMAL	14	0.0	1.3	0.0	0.6
NORMAL	15	0.0	5.3	0.0	1.7
NORMAL	16	0.0	1.4	0.0	0.4
NORMAL	17	0.0	2.2	0.0	1.0
NORMAL	18	0.0	2.0	0.0	0.0
NORMAL	19	0.0	3.0	0.0	0.4
NORMAL	20	0.0	2.3	0.0	1.3
NORMAL	21	0.0	3.9	0.0	0.6
NORMAL	22	0.0	8.2	0.0	1.3
NORMAL	23	0.0	4.0	0.0	0.8
NORMAL	24	0.0	4.3	0.0	0.7
NORMAL	25	0.0	9.1	0.0	0.6
NORMAL	26	0.0	5.9	0.0	0.2
NORMAL	27	0.0	20.6	0.0	6.0
NORMAL	28	0.9	2.2	0.9	0.7
NORMAL	29	1.4	5.0	0.0	1.0
NORMAL	30	1.4	3.5	1.4	1.2
NORMAL	31	1.9	10.1	3.9	1.0
NORMAL	32	2.1	3.3	0.0	6.3
NORMAL	33	2.8	1.5	0.0	0.0
NORMAL	34	4.1	6.9	6.6	0.8
NORMAL	35	4.2	0.0	5.4	0.0
NORMAL	36	11.0	1.2	5.6	0.0
BLADDER CA	37	0.0	0.0	0.0	0.0
BLADDER CA	38	36.7	1.6	0.0	0.0
BLADDER C	39	0.0	0.0	0.0	0.0
COLON CA	40	8.8	8.9	8.6	7.0
COLON CA	41	18.2	28.4	20.8	24.3
COLON CA	42	18.1	28.6	19.5	17.9
COLON CA	43	14.2	11.6	15.5	8.1
COLON CA	44	9.5	12.8	13.3	6.8
COLON CA	45	5.1	6.4	4.1	0.9
COLON CA	46	4.9	3.7	5.1	2.4

FIG. 3.2

SAMPLE	SAMPLE #	ANTIBODY COMBINATIONS 302-22 302-18	302-33 107-7	302-29 302-18	302-29 107-7
COLON CA	47	30.8	28.3	65.3	27.3
COLON CA	48	96.2	17.5	82.4	20.2
COLON CA	49	3.3	4.7	0.0	0.0
COLON CA	50	10.1	11.7	8.3	10.3
COLON CA	52	2.4	5.7	64.7	0.0
COLON CA	53	6.7	5.1	5.5	0.5
COLON CA	54	5.1	6.0	1.3	1.8
COLON CA	55	3.9	13.1	7.1	2.3
COLON CA	56	62.4	9.6	52.4	5.8
COLOREC CA	57	14.0	58.2	15.2	41.3
ENDOMETRIUM C	58	7.6	10.3	106.0	6.8
ENDOMETRIUM C	59	2.7	4.7	1.8	1.9
ENDOMETRIUM C	60	7.9	9.4	8.2	7.1
LUNG CA	61	10.0	13.4	10.7	9.3
LUNG CA	62	9.5	11.9	11.0	7.9
LUNG CA	63	11.3	19.0	13.5	16.2
LUNG CA	64	6.5	16.7	8.5	7.8
LUNG CA	65	12.6	20.8	14.9	11.0
OVARY CA	66	14.3	21.1	17.4	16.9
OVARY CA	67	7.0	16.4	9.9	8.9
OVARY CA	68	8.9	11.6	11.5	8.3
PROSTATE CA	69	11.4	12.7	13.8	10.8
PROSTATE CA	70	2.0	4.9	2.5	2.8
PROSTATE CA	71	6.4	0.0	9.3	3.4
PROSTATE CA	72	5.4	15.4	6.3	7.0
PROSTATE CA	73	2.2	0.0	1.6	0.0

FIG. 4

TISSUE TYPE	ASSAY 1*	ASSAY 2**	ASSAY 3***
Breast normal 90-247	NT#	500	1250
Breast normal 90-252	7574	2705	5024
Breast normal 90-254	NT	1513	2789
Breast normal 90-264	NT	0	1685
Breast normal 90-268	139	NT	432
Breast cancer 90-256	438	NT	2750
Breast cancer 90-275	2000	NT	9429
Breast cancer 90-287	20222	7333	8600
Cervix normal 90-279	2500	NT	12571
Cervical cancer 90-8083	12666	NT	70680
Colon normal 90-253	1009	NT	1689
Colon cancer 90-250	1450	NT	4275
Kidney normal 90-259	4250	NT	4275
Kidney cancer 90-289	2407	NT	5796
Liver normal	2154	614	202
Liver normal 90-451	NT	131	420
Liver cancer	2227	0	932
Met liver 90-403	NT	300	1133
Lung normal 90-248	4391	NT	6636
Lung normal 90-246	4200	NT	10000
Lung normal 90-107	NT	4166	388
Lung normal 90-118	NT	650	1200
Lung cancer 90-095	NT	5357	16077
Lung cancer 90-121	NT	>12000	40771
Ovarian cancer	8621	6517	2760
Ovarian cancer 90-260	6900	NT	20680
Ovarian cancer 90-291	2768	NT	5750
Ovarian cancer 90-291	NT	10909	14454
Uterine cancer 90-277	6574	NT	70684
Uterus normal 90-295	6574	NT	41444
average normal	3447	1284	5759
average cancer	9442	7069	26321

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* Assay 1 is 107.7 solid phase and 307.33 soluble phase.
** Assay 2 is 107.7 solid phase and 302.29 soluble phase.
*** Assay 3 is 302.18 solid phase and 302.22 soluble phase.
# NT means not tested.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/06160

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C12N15/11; C12Q1/68; C07K13/00
A61K31/70; A61K37/02; A61K39/395; G01N33/577

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C12Q ; C07K ; A61K G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	J. CELL BIOL. vol. 116, no. 6, March 1992, ROCKEFELLER UNIVERSITY PRESS,NY,US; pages 1303 - 1317 C.H. YANG ET AL. 'NuMA: An unusually long coiled-coil related protein in the mammalian nucleus' cited in the application	9,10
Y	see page 1304, right column, line 55 - page 1305, left column, line 27; figure 7	11-34, 36-38

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
06 OCTOBER 1993	15. 10. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HORNIG H.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	J. CELL BIOL. vol. 116, no. 6, March 1992, ROCKEFELLER UNIVERSITY PRESS, NY,US; D.A. COMPTON ET AL. 'Primary structure of NuMA, an intranuclear protein that defines a novel pathway for segregation of proteins at mitosis' cited in the application	9,10
Y	see page 1396, left column, paragraph 3 - right column, paragraph 1; figure 8	11-34, 36-38
Y	J. CELL BIOLOGY vol. 115, 1991, ROCKEFELLER UNIVERSITY PRESS, NY,US; page 314A T.E. MILLER ET AL. 'Release of nuclear matrix proteins during apoptotic cell death' abstracts of papers presented at the thirty-first annual meeting of the american society for cell biology, Boston, Massachusetts, US; december 8-12,1991; Abstract no.1822; abstract	11-34, 36-38
Y	CANCER RESEARCH vol. 52, no. 2, 15 January 1992, WARVERLY PRESS,INC.,BALTIMORE,MD,US; pages 422 - 427 T.E. MILLER ET AL. 'Detection of nuclear matrix proteins in serum from cancer patients' see page 425, right column, paragraph 4 - page 426, right column, paragraph 2	11-34, 36-38
A	WO,A,8 703 910 (MASSACHUSETTS INSTITUT OF TECHNOLOGY) 2 July 1987 cited in the application the whole document	-
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